COMPLEXATION AND CYCLODEXTRINS

Gerold Mosher
Diane O. Thompson
CyDex, Inc., Overland Park, Kansas, U.S.A.

INTRODUCTION

Complexation is one of several ways to favorably enhance the physicochemical properties of pharmaceutical compounds. It may loosely be defined as the reversible association of a substrate and ligand to form a new species. Although the classification of complexes is somewhat arbitrary, the differentiation is usually based on the types of interactions and species involved, e.g., metal complexes, molecular complexes, inclusion complexes, and ion-exchange compounds. Cyclodextrins (CDs) are classic examples of compounds that form inclusion complexes. These complexes are formed when a "guest" molecule is partially or fully included inside a "host" molecule e.g. CD with no covalent bonding. When inclusion complexes are formed, the physicochemical parameters of the guest molecule are disguised or altered and improvements in the molecule's solubility, stability, taste, safety, bioavailability, etc., are commonly seen.

CDs form inclusion complexes with many different types of compounds, thus their potential as formulation additives has been investigated for over 40 years. They were discovered in 1891 when Villiers (1) observed crystallization occurring in a bacterial digest of starch. Schardinger's (2) evaluation of the unusual crystalline dextrins in 1903 suggested their cyclic nature but their complete structural definition did not occur until the 1940s (3, 4). This coincided with the identification of the enzyme responsible for their production (*Bacillus macerans amylase*, now referred to as cyclodextrin glucosyltransferase: CGTase: EC 2.4.1.19), and the recognition of the complexing properties of the CD cavity. In the next 30–40 years, extensive work resulted in the ability to produce each of the parent CDs in bulk quantities.

With improvements in the cost and availability of the parent CDs came increases in the volume of scientific investigation. Limitations in the pharmaceutical utility of the CDs were becoming known and derivatives were prepared with the goal of improving characteristics such as complexing ability, solubility, and safety.

Biennial international conferences (5) and reviews (6, 7) have presented the latest research in the production, characterization, and utilization of CDs in biomedical products, foods, and cosmetics. A literature search of the 19-year period from 1967 to 1985 yields approximately 400 journal references describing CDs in pharmaceutical applications. Uekama and Otagiri (8) reviewed over half of this published literature in 1986. A search of the literature since 1986 shows that journal references have more than tripled and the patent literature has continued to grow rapidly (Fig. 1). Scientific articles have established the research applications of CDs but it is the patents that have shown the increasing interest in the commercial protection of CDs in pharmaceutical products.

The commercial viability of a CD formulation has been established with the marketing of 20 products (Table 1). Two of these products are currently on the market in the United States; one product (oral and parenteral formulations) containing a derivatized CD and the other containing α-CD. Numerous clinical trials using CD formulations have, however, been conducted or are in progress in the United States, with at least one other NDA under review.

Increasing numbers of pharmaceutical products are reaching the market place as CD formulations and research studies exploring their applications are growing exponentially. Nevertheless, the routine use of CDs in formulations is still questioned. The reluctance to develop a CD formulation is mainly due to the uncertain regulatory acceptance of a formulation containing a "nonstandard" inactive ingredient.

INCLUSION COMPLEXATION AND CDs

CDs are cyclic oligosaccharides containing 6, 7, or 8 glucopyranose units, referred to as α-, β-, or γ-CD, respectively. Each glucose unit contains two secondary alcohols at C-2 and C-3 and a primary alcohol at the C-6 position, providing 18–24 sites for chemical modification and derivatization (Fig. 2). Numerous derivatives have been prepared and described in the literature, but because of all the possible derivatives and positional and regioisomers, appropriate nomenclature must be used. The nomenclature should include at a minimum, the parent CD (α, β, or γ-CD) and the type and number of

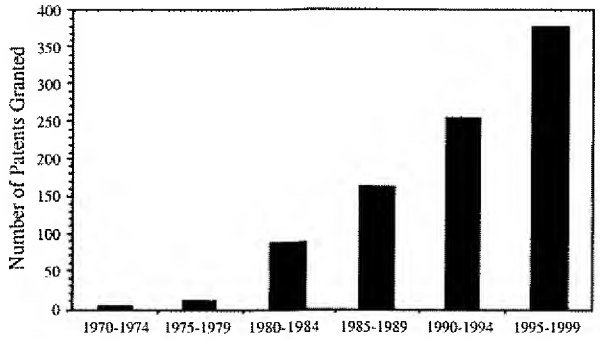

Fig. 1 Patents and patent applications in the use of CDs in pharmaceuticals. (Data collected from *Chemical Abstracts* 1967–1999.)

substituents. The substituents are usually noted by an abbreviation placed before the parent CD. Further description of the substituent group can be included with the abbreviation, if needed. For example, the hydroxyl group on the hydroxypropyl substituent can exist at one of three carbons in the propyl chain. This isomeric position is noted by a number preceding the HP notation and enclosed in parentheses. The most commonly occurring HP derivative is the (2HP)-β-CD, which is often referred to as HP-β-CD.

The number following the abbreviation of the substituent indicates the average number of substituents on the molecule, rounded to the nearest whole number. For example, HP4-β-CD indicates a β-CD with an average of four hydroxypropyl substituents. This is referred to as the molar degree of substitution (MS). It is simply a statement of the molar ratio of substituent to CD. The four substituents might be located on four different hydroxyl functionalities around the CD ring or, at the other extreme, one hydroxyl might be derivatized with a chain of four HP units. Adding further confusion is the fact that the MS can refer to the average molar degree of substitution around the CD ring, or only around each glucose ring. The latter interpretation is a carry-over from traditional carbohydrate chemistry where the lengths of the glucose chains (i.e., the molecular weights) were often not well characterized.

A similar term, the degree of substitution (DS) describes the average number of hydroxyls derivatized. Unfortunately, it has also been used to describe both the average number of hydroxyls around the CD ring and the average number around each glucose ring. A clarifying proposal to use RS (ring substituents) to designate the average number of hydroxyls derivatized in the entire CD ring and DS, the number around each glucose ring, has not been broadly embraced. The term MS will be used here and will denote the average molar degree of substitution around the CD ring.

Regardless of which convention is used for degree of substitution, none provide any indication of the position of these substituents on the glucopyranose units. If it is known, the position of the substituent on the glucopyranose unit is indicated by a number preceding the substituent abbreviation. 6-SBE1-β-CD describes the monosubstituted sulfobutyl ether derivative with the substituent attached at one of the C-6 positions. More often than not, the substituent is introduced in a random reaction process such that introduction occurs with some defined distribution at the 2-, 3-, and/or 6-positions. For these preparations, no number precedes the substituent abbreviation. HP4-β-CD implies a tetra-substituted hydroxypropyl preparation with substituents randomly distributed over all three positions of the seven glucopyranose units. A number of common CDs and their nomenclature are given in Table 2.

The 3D structure of the parent CD provides a cavity (Fig. 3) that is hydrophobic relative to an aqueous environment. The sequestration of hydrophobic drugs inside the cavity of the CD can improve the drug's solubility and stability in water, the rate and extent of dissolution of the drug:CD complex, and the bioavailability of the drug when dissolution and solubility are limiting the delivery. These properties of the CDs enable the creation of formulations for insoluble drugs typically difficult to formulate and deliver with more traditional excipients. Numerous CDs that have different complexing abilities are available. A quantitative determination of their complexing properties is necessary for proper evaluation.

CDs form inclusion complexes with hydrophobic drugs through an equilibrium process (Fig. 4), quantitatively described in Equation (1) by an association or stability constant ($K_{a:b}$),

$$K_{a:b} = \frac{[Drug_a CD_b]}{[Drug]^a [CD]^b} \quad (1)$$

where a and b represent the molar ratio of the sequestered drug molecule to the CD. The magnitude of this associate constant can be used to compare the binding effectiveness of different CDs. Various complexes with different ratios of drug-to-CD molecules can be formed, depending on the type of CD used and the size and physicochemical characteristics of the drug molecule. In dilute solutions and/or if the drug fits entirely into the CD cavity, a 1:1 complex results. However, if the cavity is large enough, two drug molecules may be accommodated, resulting in the formation of a 2:1 complex. Conversely, if the drug is

Table 1 Commercial pharmaceuticals with CD-based formulations

Drug product	Trade name	Company	Country
PGE_1/α-CD	Prostandin	Ono	Japan
Intra-arterial infusion	Prostavasin	SchwarzPharma	Germany
			Italy
Intracavernous injection	Edex	SchwarzPharma	USA
Cefotiam Hexetil HCl/α-CD	Pansporin T	Takeda	Japan
Tablet			
Piroxicam/β-CD	Various	Various	Belgium
Tablet			Brazil
Suppository			France
Oral liquid			Germany
			Italy
			The Netherlands
			Scandinavia
			Switzerland
Dextromethorphan/β-CD	Rynathisol	Synthelabo	Italy
PGE_2/β-CD	Prostarmon E	Ono	Japan
Sublingual tablet			
Benexate/β-CD	Ulgut	Teikoku	Japan
Capsule	Lonmiel	Shionogi	
Iodine/β-CD	Mena-Gargle	Kyushin	Japan
Gargling solution			
Dexamethasone Glyteer/β-CD	Glymesason	Fujinaga	Japan
Ointment			
Nitroglycerin/β-CD	Nitropen	Nippon Kayaku	Japan
Sublingual tablet			
Cephalosporin ME 1207/β-CD	Meiact	Meiji Seika	Japan
Tablet			
Nimesulide/β-CD	Nimedex	Italfarmaco	Italy
Tablet	Mesulid Fast	Novartis	Switzerland
		Boehringer Mannheim	Germany
Tiaprofenic acid/β-CD	Surgamyl	Roussel-Maestrelli	Italy
Tablet			
Chlordiazepoxide/β-CD	Transillium	Gador	Argentina
Tablet			
Omeprazol/β-CD		Hexal	Germany
Capsule			
OP-1206/γ-CD	Opalmon	Ono	Japan
Tablet			
Chloramphenicol/Me-β-CD	Clorocil	Oftalder	Portugal
Eye drop			
Cisapride/HP3-β-CD	Prepulsid	Janssen-Cilag	Belgium
Suppository			
Diclofenac/HP3-β-CD		Ciba Vision	Switzerland
Eye drop			
Ziprasidone/SBE7-β-CD	Zeldox	Pfizer	Sweden
Intramuscular injection			USA (NDA pending)
Itraconazole/HP3-β-CD	Sporanox	Janssen	USA
Oral/i.v. solution			Belgium

(Adapted from Ref. 6.)

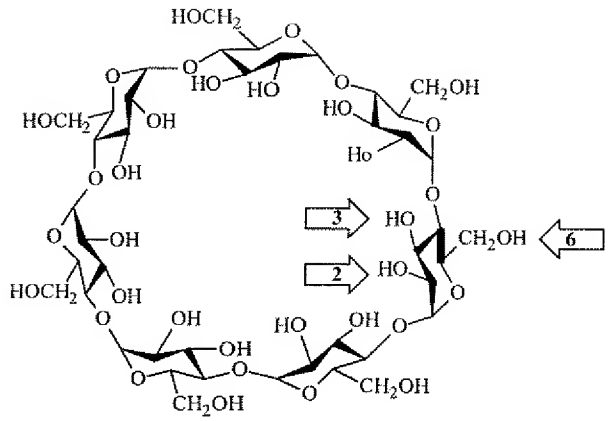

Fig. 2 Chemical structure of β-CD. Arrows indicate the 2-, 3-, and 6-hydroxyls of a glucopyranose unit. (Adapted from Ref. 6.)

very large, then several CD molecules might enclose the drug for the formation of 1:2 or higher order complexes. Although each complex has a finite stoichiometry, more than one complex may be formed in a given system. Depending on the method used to determine the association constant, it is possible to obtain a description of the stoichiometry of the complex (*a*:*b*).

Evaluating Inclusion Complexation

One of the most common methods of determining association constants and stoichiometry is the phase solubility technique (9). The technique involves adding an equal weight (in considerable excess of its normal solubility) of the compound to be complexed into each of several vials or ampoules. A constant volume of solvent is added to each container. Successively increasing portions of the complexing agent are then added to the vessels. The vessels are then closed and the contents brought to solubility equilibrium by prolonged agitation at constant temperature. The solution phases are then analyzed for total solute content. A phase diagram is constructed by plotting the molar concentration of dissolved solute, found on the vertical axis, against the concentration of complexing agent added on the horizontal axis. Two general types of phase solubility profiles are generated; Type A where soluble complexes are formed, and Type B where complexes of limited solubility are formed.

In Type A diagrams, an increase in solubility of the compound occurs as the amount of complexing agent increases. Soluble complexes are formed between the compound and the complexing agent, thereby increasing the total amount of compound in solution. Depending on the nature of the complexes formed, the diagram can be linear, A_L, or show curvature in a positive, A_P, or negative, A_N, fashion (Fig. 5). Linear diagrams are formed when each complex contains only one molecule of complexing agent. When more than one molecule of complexing agent is found in the complex, an A_P-type diagram is formed. A_N diagrams are uncommon but may result if self-association is present or high concentrations of complexing agent cause alterations in the nature of the solvent.

Type B diagrams are observed when complexes of limited solubility are formed. In Fig. 5, the segment *xy* in curve B_S shows the formation of a complex that increases the total solubility of the compound. This is similar to a Type A diagram. At point *y*, however, the solubility of the complex is reached and as additional compound goes into solution, some solid complex precipitates. At point *z*, all of the excess solid compound added to the vials has been consumed by this process. Further addition of complexing agent beyond point *z* results in depletion of the compound from *solution* by complex formation. Curve B_I is interpreted in a similar manner except that the complex formed is so insoluble that no increase in solubility is observed.

The stoichiometry of the complexes can often be determined from the ascending and descending portions of these diagrams if certain assumptions can be made (9). If a 1:1 complex is formed, the association constant $K_{a:b}$ can be determined from the slope of the initial linear portion of the phase solubility curve, and the intrinsic solubility of the compound, S_0, using Equation (2):

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \tag{2}$$

Additional methods are available to determine these association or stability constants including spectroscopy [UV (10, 11), fluorescence (12, 13), NMR (14), and ORD-CD (15, 16)], potentiometry (17), microcalorimetry (18, 19), surface tension (20), membrane permeation (21), electrophoresis (22, 23), and freezing point depression (24). Chromatographic methods include HPLC (25), paper (26, 27), and TLC (28, 29) techniques.

The binding constants obtained by different methods often correlate. For example, diazepam forms a complex with β-CD with an association constant of 220 or 208 M^{-1} as determined by phase solubility (30) vs. circular dichroism (31). There is a close correlation of the binding constants (32) for bendrofluazide and cyclopenthiazide as determined by the phase solubility method (56 and 165 M^{-1}) and UV method (60 and 178 M^{-1}).

However, while the above methods gave similar results, the association of β-CD and FCE24578 (17), a synthetic immunomodulator, exhibits a binding constant of 690 M^{-1}

Table 2 Nomenclature and substituent structures for modified CDs

	Position of substituent	Substituent structure[a]	Nomenclature #[b]-XYZ[c]#[d]-CD[e]
Parent cyclodextrins			
Alpha-CD		-OH	α-CD
Beta-CD		-OH	β-CD
Gamma-CD		-OH	γ-CD
Modified cyclodextrins neutral			
Methyl derivatives			
Dimethyl	2, 6-	$-O-CH_3$	2,6-DM14-CD
Methyl	Random	$-O-CH_3$	M#-CD
Trimethyl	2, 3, 6-	$-O-CH_3$	2,3,6-TM-CD
Ethyl derivatives	Random	$-O-CH_2-CH_3$	E#-CD
Hydroxyalkyl derivatives			
2-hydroxyethyl	Random	$-O-CH_2-CH_2OH$	(2HE)#-CD
2-hydroxypropyl	Random	$-O-CH_2-CHOH-CH_3$	(2HP)#-CD or HP#-CD
3-hydroxypropyl	Random	$-O-CH_2-CH_2-CH_2OH$	(3HP)#-CD
2,3-dihydroxypropyl	Random	$-O-CH_2-CHOH-CH_2OH$	(2,3-DHP)#-CD
Modified cyclodextrins anionic			
Carbon Based Derivatives			
Carboxy	6-	$-CO_2M$	6-C#-CD
Carboxyalkyl			
Carboxymethyl	Random	$-O-CH_2-CO_2M$	CM#-CD
Carboxyethyl	Random	$-O-CH_2-CH_2-CO_2M$	CE#-CD
Carboxypropyl	Random	$-O-CH_2-CH_2-CH_2-CO_2M$	CP#-CD
Carboxylmethyl ethyl	2,6-; 3-	$-O-CH_2-CO_2M$; $-O-CH_2-CH_3$	CME#-CD
Sulfur Based Derivatives			
Sulfates	2,6-random	$-O-SO_3M$	S#-CD
Alkylsulfates	6-	$-O-(CH_2)_{11}-O-SO_3M$	SU#-CD
Sulfonates	6-	$-SO_3M$	6-SA#-CD
Alkylsulfonates			
Sulfoethyl ether	Random	$-O-(CH_2)_2-SO_3M$	SEE#-CD
Sulfopropyl ether	Random	$-O-(CH_2)_3-SO_3M$	SPE#-CD
Sulfobutyl ether	Random	$-O-(CH_2)_4-SO_3M$	SBE#-CD

[a]M: Cation
[b]Numbers represent position of substituents if known; if the preparation is a random distribution, then no notation implies an undefined distribution at the 2-, 3-, and 6-positions.
[c]Letters represent abbreviated notation of substituent.
[d]Numbers represent the average MS rounded to the closest whole number.
[e]Indication of parent CD structure, i.e., α-CD.
(Adapted from Ref. 6.)

by a phase solubility determination but a binding constant over four times higher with a UV method. This discrepancy is due to the fact that higher order complexes contribute to spectral changes and these have not been accounted for in the calculation of the UV association constant. Inconsistencies are also observed between binding constants determined by fluorescence intensity and HPLC methods in the binding of estradiol, ethinylestradiol, and estriol, to β- and γ-CD (12). The fluorometric association constants with β-CD were lower than the corresponding HPLC values, whereas for the

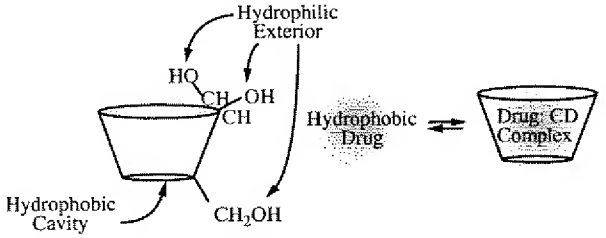

Fig. 3 Complexation of drugs inside the hydrophobic cavity of CDs.

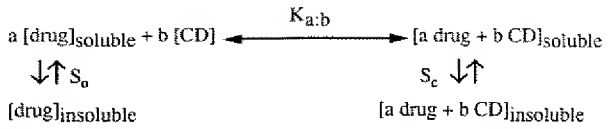

Fig. 4 Equilibrium process describing the interaction between a CD and an insoluble drug molecule to form a soluble or insoluble complex. (Modified from Ref. 6.)

complexes with γ-CD the results of both methods coincide.

pH conditions have also been observed to exert a unique effect on one method and not the other. Doxorubicin (33) and γ-CD form a complex with a $K_{1:1}$ of 617 and 718 M^{-1} as measured at pH 10 by UV and circular dichroism. This close correlation was not observed when the measurements on doxorubicin where conducted at pH 7. At pH 7, the binding constant for doxorubicin (34) was 235 M^{-1} as measured by UV but was 977 M^{-1} as measured by circular dichroism. Under a given set of conditions, a drug has only one binding constant. Therefore, this difference is reflecting how the ionization state of the drug affects the analytical measurements.

In general, binding constants can be used as an indicator of differences in binding only if the methods or conditions for determining the constants are equivalent or unaffected by the conditions. And although determining values by phase solubility might be appropriate for formulation studies, it is probably not appropriate for determination of true thermodynamic values due to the concentrations involved.

Factors Affecting Complexation

Steric effects

Cyclodextrins are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity. Complex formation with molecules significantly larger than the cavity may also be possible in such a way that only certain groups or side chains penetrate into the carbohydrate channel. The three natural CDs, α, β, and γ, have different internal diameters and are able to accommodate molecules of different size. Cyclohexane is able to complex with all three CDs, but because of size naphthalene does not complex with α-CD, which has the smallest cavity. Anthracene fits only into γ-CD, which has the largest cavity.

Derivatization of the hydroxyls on one or both faces of the natural CD can impact the steric requirements for an acceptable guest molecule. The presence of bulky groups can sterically block entrance to the CD cavity. However,

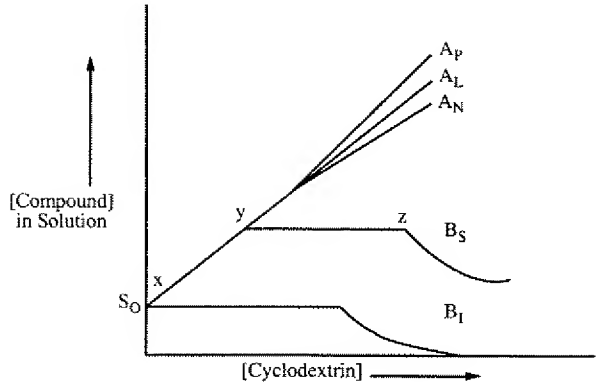

Fig. 5 Possible types of phase solubility diagrams.

some groups, depending on their number, flexibility, and position of attachment, may actually act to extend the cavity and provide for better complexation. Substitution at the 3- and 6-positions will be more likely to narrow the cavity opening while substitution at the 2- and 6-positions may allow for extension of the opening. The binding constants of flurbiprofen (35), bromazepam (36), and nitrazepam (37) to 2,6-DM-β-CD (methyl substituents in the 2- and 6-positions) are 2.3-, 2.9-, and 3.8- fold higher, respectively, than their binding to β-CD. Binding to 2,3,6-TM-β-CD (methyl substituents in the 2-, 3-, and 6-positions), however, shows constants that are less than half of that observed with β-CD.

The numbers of substituents added to the ring (MS) can also affect binding in both positive and negative manners. Müller and Brauns (38) showed that increasing the MS from 3 to 11 decreased the solubilization of hydrocortisone from 10.98 to 5.76 mg/ml for a 0.04 M HE-β-CD (hydroxyethyl) solution (~5% w/v). A similar effect was observed for digitoxin, diazepam, and indomethacin. The decrease in solubilization was thought to be due to steric hindrance of the increased number of HE substituents. An additional explanation may be that some polymerization of the HE groups may have occurred during preparation of the higher MS products, thereby creating bulkier side chains that may have crowded the cavity entrance.

The hydroxypropyl substituent, being larger yet, appears to require a lower DS to improve binding without sterically obscuring the cavity entrance. Müller and Brauns (38) have studied the effect of the DS on complexing ability (Table 3) and have observed that lower degrees of hydroxypropyl substitution (2 to 5) are more conducive to complexation. As the DS increases, the solubilization of six different drugs decreases but when the DS is from 4 to 8 the solubilization is fairly consistent.

Table 3 Effect of degree of substitution on complexation of drugs by HP-β-CD

Drug	Solubility of drug in HP-β-CD solutions[a,b] at 25°C, pH 7.4			
	MS = 2.03	MS = 4.83	MS = 7.84	MS = 8.47
Digoxin[a]	13.12	6.39	3.76	3.70
Digitoxin[a]	8.06	4.51	1.96	2.36
Levocabastin[b]	2.20	0.45	0.31	0.09
Indomethacin[b, c]	6.93	8.12	6.63	8.57
Hydrocortisone[b]	19.03	13.43	10.46	10.38
Diazepam[b]	0.72	0.67	0.44	0.46

[a]5% HP-β-CD solution.
[b]10% HP-β-CD solution.
[c]pH 7.4: Indomethacin in ionized state.
(Adapted from Ref. 39.)

There is a compromise between the steric hindrance of a substituent and its ability to extend the hydrophobic cavity. Yoshida et al. (40) have shown that introduction of the 3-hydroxypropyl (3HP) substituent (—O—CH_2—CH_2—CH_2—OH) at an MS of ~6 results in higher binding constants than those observed with β-CD, apparently due to the extension of the hydrophobic cavity. The introduction of an equivalent number of 2,3-dihydroxypropyl (2,3-DHP) substituents (—O—CH_2—CH(OH)—CH_2—OH), however, results in a decrease in the binding constants. This was speculated to be due to steric hindrance of the larger 2,3-DHP substituent, though this group, being more hydrophilic than 3HP, may not serve to extend the hydrophobicity of the cavity.

There is also a compromise between the ability to form complexes and the intrinsic water solubility. Rao et al. (41) have shown that increasing the DS of (2HP)-β-CD improves the aqueous solubility but impairs the complexation capability. Fig. 6 shows this affect for the complexation of phenolphthalein.

Greater steric interferences would be anticipated for the bulkier charged sulfoalkyl ether groups, but have not been observed. Kano et al. (42) evaluated the use of sulfopropyl ether (SPE) derivative of β-CD to interact with naphthalene. Higher association constants were observed for the SPE3-β-CD (K = 2100 M^{-1}) and SPE5-β-CD (K = 1800 M^{-1}) than were observed for β-CD (K = 730 M^{-1}). Similar results were observed for the undecylsulfated methyl CD described by Menger and Williams (43). The lack of a steric hindrance by this highly substituted ionic derivative was explained through a "micellar" arrangement of the ionic substituents. The derivative was described as a "micellar" CD because the long hydrophobic alkyl groups in the substituent are expected to align themselves to reduce interactions with the aqueous environment similar to micelle formation. The anionic charge at the end of the alkyl chain is expected to repel adjacent substituents effectively maintaining an opening to the CD cavity. Although the substituents are long enough to bend into the cavity, this is not expected due to the hydrophilic character of the ionic sulfate, which would prefer to interact with the aqueous solvent. The

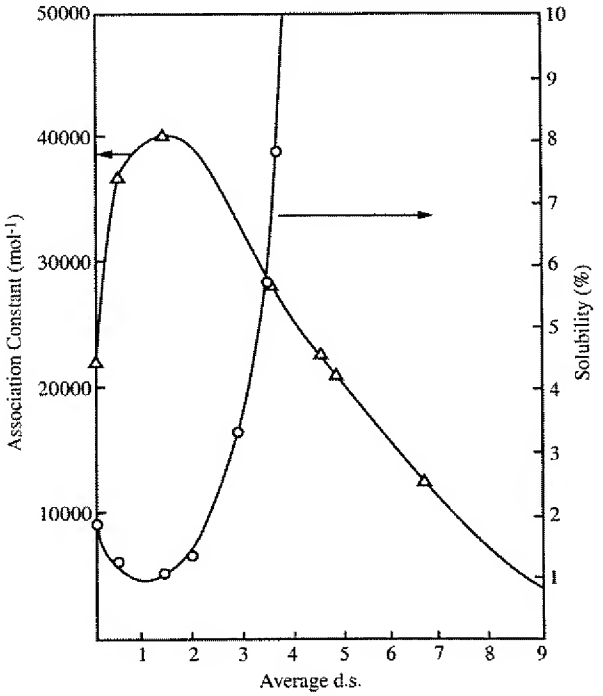

Fig. 6 Effect of changing the DS of 2HP-β-CD on its solubility (O) and the association constant (Δ) with phenolphthalein. (From Ref. 41.)

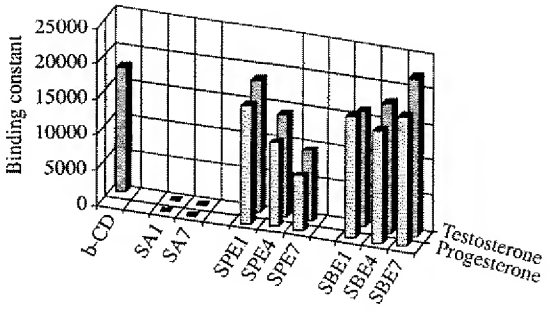

Fig. 7 Comparison of the binding constants of hydrophobic steroids, testosterone, and progesterone with β-CD and anionic β-CD derivatives. SA: Sulfonate anion at the 6-position, SPE: Anionic sulfopropyl ether substituent; and SBE: Anionic sulfobutyl ether substituent. (Adapted from Ref. 6.)

authors suggested the interaction of naphthalene occurred with the hydrophobic "arms" of the side chain and not the CD cavity although interaction with the cavity was not ruled out.

The sulfopropyl and sulfobutyl ether derivatives (44) have been further evaluated for their complexation with testosterone and progesterone (Fig. 7). Even though increasing the DS should produce more steric hindrance to complexation, the mono, tetra, and hepta substituted sulfobutyl ether (SBE1, 4, 7) derivatives all displayed comparable binding abilities for the steroids and the strength of binding was similar to that observed for β-CD. The SBE substituent behaves similar to that proposed for the undecyl sulfate CD, however, complexation with the SBE-β-CDs involves the CD cavity, as well as the hydrophobic butyl side arms.

Electronic effects

Effect of proximity of charge to CD cavity: The ionic derivatives that have charges closest to the CD cavity are the carboxylate, sulfate, and sulfonate derivatives. The complexation characteristics of the directly carboxylated CDs, C-β-CDs have not been reported but the highly anionic sulfated CD derivative (S14-β-CD) does not appear to form inclusion complexes (45). This may be either due to steric effects from the 14 sulfate substituents or due to the ionic state of the CD.

The effects of charge proximity on CD complexation behavior were evaluated (Fig. 7) by studying the complexation of two steroids by the sulfonate, sulfopropyl ether (SPE), and sulfobutyl ether (SBE) derivative (44). Electronic effects seem to be more of a factor than steric effects because even when only one sulfonate substituent is attached at the 6-position, (6-SA1-β-CD) the derivative loses its complexation capability. The binding constant for testosterone is only 64 M^{-1} for 6-SA1-β-CD versus 17,800 M^{-1} for the neutral β-CD. The attachment of a single negative charge close to the CD cavity appears to disrupt the thermodynamics driving the complexation.

When one sulfonate ion (SA1) is directly attached to the CD, there is a minimal binding of the steroids but as the charge is spaced away by the three carbon propyl (SPE1) or a four carbon butyl group (SBE1), the derivatives regain the binding capability of the β-CD molecule. The monosubstituted sulfopropyl and sulfobutyl derivatives (SPE1 and SBE1) are able to bind progesterone and testosterone as well as β-CD. This suggests that ionic substituents too close to the CD cavity adversely disrupt the thermodynamics driving the inclusion complexation. Moving the charge away from the cavity re-establishes the complexation characteristics but this is dependent on the charge density in the structure.

Effect of charge density: As the charge density increases in the sulfopropyl family from a mono to a tetra and hepta anion, the binding of the steroids decreases. However, when the sulfonate anion was spaced four methylene units away, the charge density did not adversely affect the binding of the steroids. The mono, tetra, and hepta substituted sulfobutyl ether derivatives all displayed comparable binding abilities for the steroids and the strength of binding was similar to that observed for β-CD.

Effect of charge state of CD and drug: Ionic CDs are capable of complexing neutral hydrophobic drugs, if the ionic charge is not directly attached to the carbohydrate backbone of the CD. The trianion of CM3-β-CD (25) is able to complex a neutral drug, hydrocortisone with an association constant that is 74% of that observed for neutral β-CD. Although this anionic derivative is less effective than the neutral β-CD, a more favorable situation has been observed for the interaction of anionic SBE-β-CDs and neutral drugs. Okimoto et al. (46) reported that the anionic SBE-β-CD (Table 4) often exhibits 1:1 binding constants with neutral drugs that are comparable to or better than those observed for the neutral HP-β-CD. The better binding may be due to the butyl "micellar" arms extending the hydrophobic cavity of the CD.

When the drug and the CD are both charged, electrostatic effects may be observed. Adverse electronic effects have been observed for the complexation between the anionic form of indomethacin and the dianion of carboxymethyl-β-CD, CM2-β-CD (38). At pH 6.6, indomethacin exists as an anion and under these conditions, the anionic carboxymethyl CD did not complex the drug at all, probably due to electrostatic

Table 4 Effect of charge state of drug on 1:1 binding to neutral HP-β-CD and anionic SBE-β-CD

	Neutral drug *Ka* (M^{-1})		Anionic drug *Ka* (M^{-1})		Cationic drug *Ka* (M^{-1})	
Drug	**HP-β-CD**	**SBE-β-CD**	**HP-β-CD**	**SBE-β-CD**	**HP-β-CD**	**SBE-β-CD**
Cinnarizine[b](46)	22,500	69,700			4,000	17,500
Cinnarizine(1:2)[b](46)	494	—			6	—
Danazol[c](47)	76,600	94,900				
Digoxin[d](48)	4,900	6,880				
Hydrocortisone[d](48)	1,340	2,150				
Indomethacin[b](46)	1,590	4,710	955	819		
Kynostatin[e](49)	95	292			20	96
Kynostatin(1:2)[c](49)	26	4			3	0
Miconazole[b](46)	104,000	417,000			42,300	410,000
Miconazole(1:2)[b](46)	45	12			11	<1
Naproxen[b](46)	1,670	3,600	331	432		
Papaverine[b](46)	337	1000			17	94
Phenytoin[b](48)	1,070	756				
Progesterone[d](48)	11,200	18,300				
Testosterone[d](48)	11,600	22,500				
Thiabendazole[b](46)	136	443			7	56
Warfarin[b](46)	2,540	10,100	509	262		

[a]Binding constants for 1:1 complexation unless noted.
[b]HP = Encapsin ™ MS = 3.5; SBE-β-CD MS = 7.
[c]HP = Roquette MS = Not reported; SBE-β-CD MS = 7.
[d]HP = Molecusol ® MS = 7–8; SBE-β-CD MS = 7.
[e]HP = Molecusol ® MS = 7–8; SBE-β-CD MS = 4.
(Adapted from Ref. 6.)

repulsions. However, the tri-anion, CM3-β-CD (25) has been reported to complex the anionic forms of warfarin and indomethacin (Table 5) although only at 71 and 60% of the binding observed for the neutral β-CD.

Experience with the carboxymethyl derivatives suggested the position of the charge in the drug structure may affect the interaction with an anionic CD. The spacing of the charge by the butyl group in the SBE substituent appears to lessen these repulsive effects observed for the shorter carboxymethyl substituent. The binding constants between the anionic forms of indomethacin, and naproxen and the anionic SBE-β-CD (Table 4) are almost equivalent to those observed for the neutral HP-β-CD. The binding constant between SBE-β-CD and the anionic warfarin molecule, however, is much lower than that with HP-β-CD, suggesting that the position of the charge in the drug and how this interacts with the charge in the CD may be important.

Table 5 Effect of charge state of drug on binding to neutral β-CD and anionic carboxylmethyl-β-CD

Drug	**Charge state of drug**	**β-CD (neutral) binding constant (M^{-1})**	**CM3-β-CD (anionic) binding constant (M^{-1})**
Hydrocortisone	Neutral	6200	4600
Indomethacin	Anionic	620	250
Warfarin	Anionic	520	150
Propranolol	Cationic	220	400

(Adapted from Ref. 25.)

Cooperative electrostatic interaction between the cationic drugs and the anionic CDs have been observed. Enhanced complexation is observed for the complexation of the cationic form of propranolol with the anionic CM3-β-CD (Table 5) and is probably due to cooperative electrostatic interactions. Similar positive interactions are observed with the SBE-β-CD and the cationic forms of cinnarizine, miconazole, papaverine, and thiabendazole (Table 4).

One difference in complexation performance of ionic versus neutral CDs is in their inability to participate in 1:2 or 1:3 complexations. The ionically charged CDs do not effectively form higher order complexes probably due to electrostatic repulsions between the first CD to sequester the drug and the incoming ionic CD. As the charge density increases, this repulsive effect is magnified. Rajewski et al. (50) demonstrated that as the charge density of the SBE-β-CD increases from one to four to seven, the solubilization of cholesterol decreases. Fortunately, the SBE-CDs are able to complex drugs effectively with the 1:1 complexation so the inability to effectively participate in 1:2 complexes does not impose any practical disadvantages.

Temperature, additives, and co-solvent effects

Inclusion complexation is an equilibrium process and the strength of association is affected by the temperature of the system. In most cases, as the temperature increases, the binding constant will decrease. For example, the binding constant for the neutral naproxen molecule (51) and β-CD decreased from 1379 to 975 to 778 M^{-1} as the temperature increased from 25°C to 35°C and 45°C, respectively. The solubility of a drug in the CD solution may increase with an increase in temperature even though the binding constant is decreasing because the increased temperature improves the intrinsic solubility of the free drug (S_O in Fig. 5) (52, 53).

Organic solvents (54–56) typically reduce the complexation of a drug with CD by competing for the hydrophobic cavity. They also reduce the solubility of most CDs and their complexes. Recently, Loftsson et al. (57) and Redenti et al. (58) have reported on the use of water soluble polymers and hydroxy acids, respectively, to increase CD:drug complexation and improve the solubilizing effect.

Release from the Complex

Complexation of drugs by CDs improves their delivery characteristics and does not interfere with their activity because complexation is a rapidly reversible process. In aqueous solution, drug:CD complexes are continually forming and dissociating with lifetimes in the range of milliseconds or less (59, 60). Although slower kinetics of dissociation are seen with stronger binding, the rates are still fast and essentially instantaneous. After administration, the drug is released from the complex upon dilution, and in some cases with contributions from competitive displacement with endogenous lipophiles, binding to plasma and tissue components, drug uptake into tissues is not available to the complex, and rapid elimination of the CD (61).

The effects of dilution are demonstrated in Fig. 8 (62) for complexes with various binding constants. Most drug:CD complexes exhibit binding constants in the range of 100–20,000 M^{-1} and Fig. 8 demonstrates that even for the more tightly bound drugs, a 1:100 dilution will reduce the percentage of drug complexed from 100% to 30%. A 1:100 dilution is readily attained for

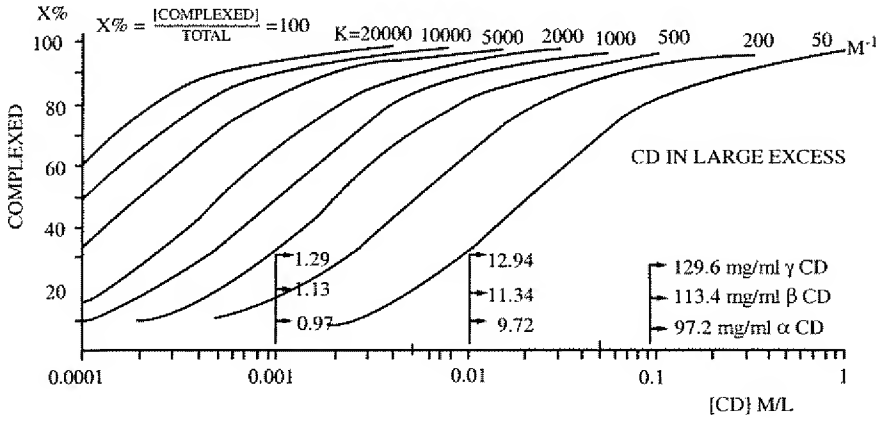

Fig. 8 Correlation between percentage of complexed drug and CD concentration at various K values. (Adapted from Ref. 62.)

intravenous products and upon dilution in the stomach and intestinal contents.

Dilution is minimal, however, when drugs are administered via routes such as ophthalmic, transmucosal, and transdermal. Under these conditions, the drug can still be displaced from the CD cavity by competing lipophiles such as triglycerides, cholesterol, bile salts, and other hydrophobic compounds often found in high concentrations at the site of delivery.

BENEFITS OF COMPLEXATION

Improvement in Solubility, Dissolution, and Bioavailability

CD formulations provide improved aqueous solubility to poorly soluble drugs, and the drug:CD complex often exhibits improved dissolution characteristics compared to other formulations of the drug. These two features can provide for an improvement in oral bioavailability when solubility and the rate of dissolution are limiting the availability of the drug for absorption. For example, the drug cefotiam hexetil hydrochloride forms a gel under the acidic conditions of the gastric contents and shows poor dissolution. A variety of excipients were screened to prevent gelation and α-CD complexation afforded the best formulation for the dissolution and solubilization of the drug (63).

Another example is the calcium channel blocker, cinnarizine. This drug exhibits a very low and erratic bioavailability after oral administration as a suspension ($F = 8 \pm 4\%$) or capsule ($F = 0.8 \pm 0.4\%$). When it was administered as a complex with SBE4-β-CD or HP-β-CD (64), either as a solution ($F = 55-60\%$) or in a capsule ($F = 38 \pm 12\%$), the bioavailability was significantly enhanced. The improvement in bioavailability was attributed to enhanced dissolution and solubilization via the complexation.

A review of the literature reveals several hundred citations and reviews that describe the effects of complexation on dissolution and bioavailability of drugs. A broad range of CD and CD derivatives have been investigated as well as many different drugs. Some other representative examples are spironolactone (65), meclizine (66), ketoprofen (67), oxazepam (68), danazol (69), phenytoin (70), and tolbutamide (71). Although these studies demonstrate the general application of complexation for improvements in dissolution and bioavailability, the use of complexation may not be practical for some dosage forms due to the amount of CDs required. β-CD for example has a molecular weight of 1135. If one uses a mole ratio of 5:1 to promote solubility, then over 350 mg of CD will be required for a 25 mg dose of a drug having a molecular weight of 400. This can limit the type and dose of drug that can realistically be used with complexing agents for solid oral dosage forms.

Solution formulations, however, do not typically have these same constraints, and complexation provides an alternative to the use of non-aqueous solvents or large volumes. A few derivatized CDs (e.g. hydroxypropyl and sulfobutyl ether) can be safely administered by parenteral routes. This is often where complexation and its improvements in aqueous solubility can be most readily utilized. The derivatized CDs often can be used to replace cosolvents such as ethanol, polyethylene glycol, and lipids, as well as provide an alternative to the use of emulsions and liposomes. The hydroxypropyl and sulfobutyl ether derivatives are stable in solution and can be readily autoclaved, often improving the heat stability of drugs. There are however, reports of complexation of CDs with anti-oxidants (72) and preservatives (73, 74) with both decreased and increased efficacy (75).

Reduction of Unpleasant Side Effects and Bitter Taste

Improvements in the rate and extent of dissolution of a drug can improve the rate of absorption of the drug. Reducing the contact time between the drug and the tissue mucosa can help minimize tissue irritation produced by drugs. Nonsteroidal anti-inflammatory drugs cause a high incidence of gastrointestinal ulcerative lesions that are a result of both local irritation from the drug and systemic inhibition of prostaglandin synthesis by the drug. CD formulations of naproxen (76), diclofenac (77), and piroxicam (78) cause fewer gastric lesions associated with the acute local tissue irritation than produced by the drug alone. Formulations containing CDs have also shown less irritation than nonCD containing formulations for ophthalmic (79), intravenous (80), and intramuscular (81) administration, and in cellular injury screening tests (82).

Complexation with CDs can also have the effect of reducing the amount of contact with taste receptors. This can be of great benefit in the preparation of oral solutions. Not only are the drugs "masked" from the receptors by inclusion in the CD cavity, but the increased hydrophilicity enables the easier removal of the bitter substance from the receptor surface as well. The apparent concentration of the uncomplexed bitter drug is a function of the complexation constant, the amount of free CD, and the water solubility of the drug (83). Complexation has been used to mask the unpleasant bitter taste of a number of drugs such as oxyphenonium

bromide (84), propantheline bromide (85), clofibrate (86), and acetaminophen (83).

Improvements in Drug Stability

CDs are normally thought of as stabilizing agents in pharmaceutical formulations (87, 88). They have been shown to stabilize drugs to hydrolysis (89) and hydrolytic dehalogenation (90), oxidation (91), decarboxylation, and isomerization (92), both in solution and in the solid state. They can, however, accelerate these same reactions (93, 94). The nature of the stabilization or destabilization depends on the CD used (parent and functional groups of any derivative) and on the position of the guest molecule inside the CD. If the molecule is positioned such that the area of instability is located outside the CD, no effect on stability may be observed. When the position allows interaction of the CD hydroxyls (or derivative functional groups) with a hydrolytically prone site, decreased stability may be observed but if the site is located fully within the CD, enhanced stability usually results.

In the solid state, stabilization of drugs to degradation has been reported for numerous drug including nicardipine (95), colchicine (96), prostaglandin E_1 (97), diclofenac (98), and sulfamethoxazole (99).

Stabilization is not limited to small compounds, as larger molecules such as peptides and proteins can also form complexes that result in enhanced chemical and physical stability (100). The CDs will typically interact with functional groups present on exposed surfaces of the macromolecules and often form multiple complexes (several CDs per molecule). Stabilization against aggregation has been observed for CD complexes in solution with proteins such as ovalbumin and lysozyme (101), carbonic anhydrase (102), and insulin (103), and in the solid state with albumin and gamma-globulin (104). CD complexes have also been investigated as chaperone-mimics (105) in the refolding of denatured proteins (106).

The degree of stabilization/destabilization of a drug complexing with a CD depends not only on the rate of degradation within the complex, but also on the fraction of drug that is complexed (88), and the stoichiometry (107). Increased stability is often observed for compounds having high association constants and those that tend to form higher order complexes.

Reduction in Volatility

Inclusion complexes have been prepared with a number of volatile substances (108, 109) including spices, flavors, essential oils, and several drugs. CD complexation has been shown to reduce the volatility and improve the stability of many compounds. Examples include lemon oil (110) and other flavoring agents (109), clofibrate (86), isosorbide 5-mononitrate (111), and nitroglycerine (112). In addition, complexation facilitates the handling of products, particularly because they transform liquids to solids. The solid form can also provide certain formulation advantages over liquids such as eliminating the melting point and hardness reduction of suppositories commonly observed when liquids are added (113).

CYCLODEXTRINS

α-, β-, and γ-CDs

CDs, also called Schardinger dextrins, cycloglucans, or cycloamyloses, are α-1,4 linked cyclic oligosaccharides obtained from enzymatic conversion of starch. The parent or natural CDs contain 6, 7 or 8 glucopyranose units and are referred to as alpha-(α-CD), beta-(β-CD), and gamma-(γ-CD) CD, respectively. The chemical structure of β-CD (Fig. 2) shows the cyclic nature of the molecule,

Table 6 Characteristics of α-, β-, and γ-CDs

	α	β	γ
No. of glucose units	6	7	8
Molecular weight	972	1135	1297
Cavity diameter, Å	4.7–5.3	6.0–6.5	7.5–8.3
Solubility @25°C (g/100 mL)			
Water	14.5	1.85	23.2
Methanol	i	i	>0.1
(Aqueous) 50%	0.3	0.3	208
Ethanol	i	i	>0.1
(Aqueous) 50%	>0.1	1.3	2.1
2-propanol	i	i	>0.1
Dimethylsulfoxide	2	35	
Propylene glycol	1	2	
Glycerin	i	4.3	
Solubility in water (g/100 g)			
20 °C	0.90	1.64	1.85
25 °C	1.27	1.88	2.56
30 °C	1.65	2.28	3.20
35 °C	2.04	2.83	3.90
40 °C	2.42	3.49	4.60
45 °C	2.85	4.40	5.85
50 °C	3.47	5.27	
55 °C		6.05	

(Adapted from Ref. 7.)

and the presence of three hydroxyl groups on each glucopyranose unit. Two of the hydroxyls are secondary alcohols and are located at the C-2 and C-3 positions of the glucopyranose unit. The third hydroxyl is a primary alcohol at the C-6 position. The hydroxyls provide the hydrophilic exterior responsible for the aqueous solubility (Table 6) of the CDs.

In three dimensions, this structure forms a truncated cone where the primary hydroxyl groups are located on one face and the secondary hydroxyl groups on the other. The interior of the cone is hydrophobic due to the presence of the glycosidic ether oxygens at O-4 and the hydrogens attached to C-3 and C-5, and thereby provides a cavity for the inclusion of hydrophobic compounds. The cavity varies in size with α-CD being the smallest at about 5.3 Å across and γ-CD the largest at 8.3 Å diameter (Table 6).

Properties in solution

The solubilities of the natural CDs in water varies and is quite dependent on temperature (Table 6). The unusually low water solubility of β-CD is due to the very rigid structure that results from the H-bonding of the C-2 hydroxyl of one glucopyranose unit with the C-3 hydroxyl of an adjacent unit (114). In the β-CD molecule, a complete set of seven intramolecular H-bonds can form, effectively limiting interactions with the solvent. This "belt of H-bonds" is incomplete in the other parent CDs thus allowing more favorable interactions between α- and γ-CD and water molecules. This is consistent with the observation of a less favorable enthalpy and entropy of dissolution (115) for β-CD versus α- and γ-CD. Recent studies have suggested that the abnormally low water solubility of β-CD may be exacerbated by aggregation of these rigid β-CD molecules (116). The solubility of β-CD can be increased by disrupting this aggregation through the addition of solvent structure-altering substances such as urea (117), inorganic salts (118), and hydrophilic polymers (119).

Solubility of the CDs is low in most organic solvents (Table 6). In aqueous/organic cosolvent systems, the solubility decreases as the organic concentration increases, with the exceptions of ethyl and propyl alcohol where a maximum is observed at around 30% alcohol (120).

In solution, the CDs are fairly stable to hydrolysis in alkaline medium. Under acidic conditions, the α-1, 4 glycosidic bonds are slowly broken to open the ring, and then to give glucose and a series of linear maltosaccharides. The initial opening of the ring is a slower process by about 2–5-fold than the subsequent hydrolysis of the linear dextrins. The initial ring-opening kinetics are the most important for pharmaceutical preparations as complexation requires the intact cyclic structure. Half-lives for the ring opening reaction step at 70°C and 0.2 *M* HCl are 25.2, 14.5, and 7.1 h for α-, β-, and γ-CD, respectively (121). Additional pH/rate data are available in the literature (122).

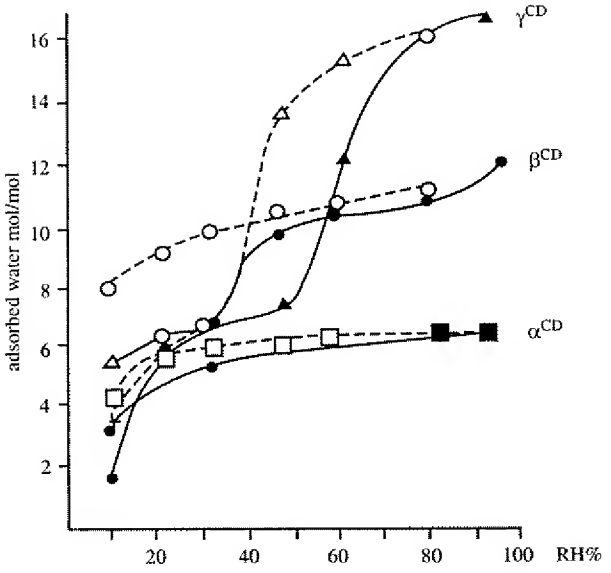

Fig. 9 Water vapor sorption isotherms for α-, β-, and γ-CD at 40 °C. (Dashed line: adsorption, solid line: desorption). (Adapted from Ref. 7.)

Similar reaction products are observed with gamma-irradiation in solution (123), but in the crystalline solid state, the mechanism appears to be different and no glucose is formed.

Solid state properties

The three natural CDs form crystalline structures in the solid state that decompose above 200°C with no definite melting points. They are not considered hygroscopic, but they do form various stable hydrates. The water vapor sorption isotherms (Fig. 9) show two phases for the β- and γ-CDs, and one phase for α-CD. At 11% RH, α-CD absorbs 4 water molecules and upon long-term storage, forms a stable hydrate with 6 water molecules. The water content gradually increases with increasing humidity to a constant value of 6.6 water molecules per CD molecule at and above 79% RH (124). Four different crystalline forms of α-CD have been reported; two forms containing approximately 6 water molecules, one form containing 7.6 water molecules and a dehydrated form.

The water content of β-CD increases with increasing humidity and passes through a plateau region at about 23–31% RH where the water content is about 5–6

water molecules. Another leveling of the plot occurs at humidities of 60–79%. Both 12-water (125) and 11-water (126) hydrated crystalline forms have been reported along with a dehydrated form. Upon standing for several weeks, the 11-water form will convert to the 12-water form, which is stable over a large range of humidity conditions.

X-ray diffraction patterns of γ-CD stored under various humidity conditions also show the existence of three distinct crystalline forms. A dehydrated form is observed at low humidities and a hydrated form containing almost 17 water molecules occurs at 93.6% RH. An intermediate crystalline form containing 7 water molecules is found at intermediate RH values which corresponds to the plateau region at 20–30% RH in the sorption isotherm. The hydrate and dehydrate forms pass through the intermediate form during dehydration, and hydration respectively (124).

CD Derivatives

Hundreds of modified CDs have been prepared and shown to have research applications. However, only the derivatives containing the hydroxypropyl (HP), methyl (M), and sulfobutyl ether (SBE) substituents are in a position to be used commercially as new pharmaceutical excipients. These substituents vary in size and electronic character and are attached to the CD structure through reaction with one or more of the three hydroxyl groups of the glucopyranose units. The parent CDs contain 18 (α-CD), 21 (β-CD), or 24 (γ-CD) hydroxyl groups that are available for modification. The most reactive hydroxyls are in the C-6 position and the C-3 hydroxyls are the least reactive. However, the difference in reactivity is not great, and changing reaction conditions can often alter the position of substitution. The preparation of homogenous, selectively derivatized CDs is, therefore, not an easy task. With all the options available for positional and regioisomers to be formed, one must be careful in describing the various derivatives. A discussion of nomenclature is provided earlier.

The main derivatives under development as excipients are all derivatives of β-CD: 1) A randomly methylated derivative with an average MS of 14 (M14-β-CD), 2) Two different 2-hydroxypropyl derivatives, one with an average MS of approximately 3 ((2HP)3-β-CD) and the other with an average MS of 7 ((2HP)7-β-CD), and 3) A sulfobutyl ether derivative with an average MS of 7 (SBE7-β-CD). Glucosyl and maltosyl CDs (127, 128) which contain a mono- (G_1-β-CD) or disaccharide (G_2-β-CD) substituent, have also been reported and show promise for the future.

Methylated

Methylation can be controlled to produce mono- to fully derivatized CDs. The introduction of the methyl substituent dramatically improves the water solubility of the derivative versus the parent CD. Aqueous solubility increases as the number of methyl groups reaches 14 and then decreases as substitution approaches 21. The 2,6-DM14-β-CD and the 2,3,6-TM21-β-CD have solubilities of 57 and 31 g/100 ml, respectively, versus 1.8 g/100 ml for the parent β-CD. The introduction of the methyl groups disrupts the "belt of H-bonds" effectively increasing the polarity of the derivative.

The aqueous solubility of these derivatives is adversely affected by temperature, however, and precipitation occurs during heat sterilization. The mixture of randomly methylated β-CD (M14-β-CD) (129), however, exhibits a favorable water solubility (>50 g/100 ml) that increases as temperature increases (130).

The extent of methylation is also important in optimizing complexation. The introduction of the methyl substituent at the 2- and 6- positions appears to improve complexation. Binding constants for 2,6-DM14-β-CD with many drugs is an average five times of that observed with β-CD. The methyl groups seem to increase the hydrophobicity of the CD cavity possibly by providing an "extension" of the cavity. Derivatization of the remaining C3 hydroxyls, however, results in a dramatic decrease in complexation ability. This may result from the distorted cyclic structure formed when the CDs are permethylated (131). The altered conformation also impacts the stability of the derivative in acidic solutions. Degradation half-lives of 2.1 and 12.0 h have been reported for a randomly methylated (2, 3, 6) M14-β-CD and a 2,6-DM-β-CD, respectively, in 1 *M* HCl at 60°C (129). Under similar conditions, β-CD has a half-life of 5.4 h.

The mixture of randomly methylated β-CD, although partially derivatized at the 3-position, still maintains the favorable binding characteristics of 2,6-DM14-β-CD. One report (129) demonstrated that M14-β-CD solubilized 26 drugs more effectively than β-CD and the extent of solubilization was on average 80% of that observed for the purified 2,6-DM-β-CD preparation.

Studies suggest that an optimal definition for a commercially viable methylated CD is the partially methylated β-CD (M14-β-CD) containing an average MS of approximately 14 with the substituents at the 2-, 3-, and 6-positions. This material is produced economically, has an aqueous solubility that increases with temperature, and has binding constants higher than those observed with the unsubstituted β-CD and close to those observed with the 2,6-DM-β-CD.

Hydroxypropyl

Hydroxy alkylation of β-CD requires treating base-solubilized β-CD with the appropriate epoxide or haloalcohol (132, 133). Propylene oxide or propylene carbonate are used in the preparation of 2-hydroxypropyl β-CD ((2HP)-β-CD), the derivative being commercialized. The reaction occurs at both primary and secondary alcohols on the β-CD generating a mixture of numerous isomeric forms (134, 135). This results in a heterogeneous product that is amorphous and highly water soluble.

The 2-hydroxypropyl derivative has been the subject of numerous clinical trials and is commercially available from several suppliers. Brandt (136), Müller (137, 138), and Pitha (139) have described its preparation and use. The DS can affect the ability of the hydroxypropyl derivatives to form complexes. It can also affect the solubility of the derivatives. The mono substituted derivative, (2HP)1-β-CD is actually less soluble than β-CD (140). However, at degrees of substitution of ~2.7 and higher, the solids are amorphous and exhibit solubilities in excess of 50% w/v (135). Water uptake by the solid forms is low. At 75% RH and 25°C, the (2HP)-β-CDs show less water uptake than the parent β-CD (135), and the water uptake decreases with increasing MS.

As discussed earlier, the need to control the DS becomes important to balance water solubility and complexation capability. Two commercial preparations of (2HP)-β-CD, Encapsin™ and Molecusol®, have recognized the need for this compromise and have substitution levels that provide a balance between solubility and complexation. Encapsin and Molecusol have MS values of approximately 3 and 7, respectively. Although both (2HP)-β-CD commercial preparations are unique, each manufacture can reproducibly generate materials to meet defined specifications. These (2HP)-β-CD derivatives appear to be equally effective in complexation and have water solubilities exceeding 50% w/v. Both have been administered parenterally.

Sulfobutyl ether

Rajewski (48) prepared the directly sulfonated CDs through the introduction of the sulfonic acid moiety at the C-6 position. These anionically charged sulfonic acid substituents were spaced away from the CD with alkyl groups by Parmerter (141) and Lammers (142) in the preparation of sulfopropyl derivatives of CDs.

Stella and Rajewski (44) later described the preparation of sulfoethyl through sulfohexyl derivatives of the CDs. The sulfonate and sulfoalkyl ether derivatives can be prepared with different average degrees of substitution (143), are isolated as the sodium salts, and demonstrate water solubilities independent of the MS. Likewise, no effect on complexation is seen with changes in MS when the alkyl spacer is butyl (Fig. 7). The SBE-β-CD derivatives are amorphous and similar to HP-β-CDs, tend to form amorphous complexes. They are highly water soluble (>50 mg/ml), and somewhat hygroscopic, reversibly picking up water at humidities below RH 60%.

The SBE7-β-CD derivative has been used in clinical trials and is being developed commercially as Captisol. It is well characterized and suitable for parenteral administration.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Oral Pharmacokinetics

The parent CDs are poorly absorbed from the gastrointestinal tract. Reported values for absorption range from 0.1 to 0.3% (144) for rats fed a diet containing 5–10% β-CD, to ~2% (145) when the doses were administered in an isolated rat ileum closed-loop experiment. When ^{14}C β-CD was administered orally, values as high as 4.8% have been reported for appearance of the label in the urine (146). This higher value was attributed to absorption of the metabolites of β-CD. The small amount of intact CDs absorbed orally probably does so by passive means (147), via the paracellular route (148). Oral absorption studies with α- and γ-CD have shown ≤2% and ≤0.1% absorption, respectively (149, 150).

The majority of an orally administered dose of α- and β-CD will be metabolized in the colon. This has been demonstrated both in rats (146) and man (151) with very little hydrolysis occurring in the upper gastrointestinal tract (GIT). Microbiological studies (152) have shown that most of the human colonic bacterial strains can degrade α- and β-CD and this activity can be stimulated by as little as 2–4 h of exposure to the CDs. The typical 40 h transit time through the human colon provides adequate time to induce the bacterial enzymes to provide for complete hydrolysis of the CDs in the colon. Likewise, most of an oral dose of γ-CD is metabolized in the GIT. However, studies with radiolabelled γ-CD suggest that most of its metabolism occurs in the upper GIT (153).

The derivatized CDs are generally more resistant to hydrolysis in the GIT than the parent CDs. Oral bioavailability of HP-β-CD in dogs is estimated at 3.3% and is less in rats, and about 60% of the dose is excreted

Table 7 Pharmacokinetic parameters for several CDs

CD	Species	$t_{1/2,\alpha}$ (min)	$t_{1/2,\beta}$ (min)	Vd_{ss} (mL/kg)	CL_T (mL/h/kg)	Ref.
β-CD	Rat	1.5–2.9	23.9–50.2	152–176	204–372	(158)
γ-CD	Rat		20			(153)
(G_1-β-CD)	Rabbit			191	283	(159)
(G_2-β-CD)	Rat	4.3	31.1	534.6	979.4	(127)
HPβ-CD	Rat		24		512	(154)
(MS = 2.7)	Dog		48		188	(154)
HPβ-CD	Human		72–108	164–240	96–126	(160)
DM-β-CD	Rat		22.7–42.3			(156, 161)
S-β-CD (MS = 9.6)	Rabbit			144	32	(159)
S-β-CD (MS = 17.6)	Rabbit			172	47	(159)
S-β-CD (MS = 13.3)	Rat			113	52	(159)
SBE-β-CD	Rat		18	300	588	CyDex unpublished
(MS = 7)	Dog		66	400	282	CyDex unpublished
	Man		84	185	114	CyDex unpublished

unchanged (154). Oral absorption in humans has not been observed (150). Oral administration of ^{14}C HP-β-CD to rats results in approximately 3% of the radiolabel appearing in the urine, 71% in the feces, and 3% being exhaled (155).

The methylated derivatives have shown somewhat greater oral absorption. The absorption of DM-β-CD has been reported as 6.3–9.6% in the rat (156), and M-β-CD as 0.5–11.5% (153).

Parenteral Pharmacokinetics

Intravenously administered CDs disappear rapidly from the systemic circulation and are excreted mainly through the kidney. α- and β-CD are excreted almost completely in their intact form, but some metabolism is observed with γ-CD. Reports vary with regard to the amount of metabolism from "substantial" (157) to 10% or less (153). The hydrophilic CD derivatives are likewise rapidly cleared following intravenous administration and most are excreted unchanged in the urine. Linear, two compartment pharmacokinetics are usually observed although the initial distribution kinetics are very rapid and may not always be captured.

The disposition parameters for several CDs are given in Table 7. The steady state volumes of distribution (Vd_{ss}) correspond well with extracellular fluid volume in each species evaluated, suggesting little or no distribution of most CDs into other tissues or storage compartments. Studies with ^{14}C HP-β-CD have shown that the small amount that does distribute, has been found mainly in the kidney and lungs of rats following single intravenous doses, and in the kidney and liver of dogs after chronic (1 month) intravenous dosing (154). The total plasma clearances (CL_T) are dose independent and are indicative of clearance at a rate comparable to glomerular filtration (158, 160). Thus, as with any compound whose elimination is closely tied to kidney function, linear pharmacokinetics may not always be observed in the presence of poor renal function.

SAFETY OF CD

Oral Safety

The oral safety of the parent β-CD was first reported in 1957, and it was erroneously suggested that the material was unsafe (162). Subsequent studies by Anderson et al. (163) and Gerlóczy (164) demonstrated that α- and β-CD produced no toxic effects when fed to rats for 30–90 days at 1% of the diet or at 1 and 2 g/kg daily doses. The odd, irreproducible results of the first report were probably due to the inconsistent purity of early CD materials and the possible presence of residual organic solvents.

Both rodent and nonrodent studies have been conducted on the parent CDs. Szejtli and Sebestyén (165) reported the parent CDs to be nontoxic at very high oral doses. Mortality was not observed, even in animals treated with

the highest possible oral doses. Therefore, the LD_{50} in rats is reported to be greater than 12.5, 18.8, and 8 g/kg body weight for α-, β-, and γ-CD, respectively.

In general, the oral administration of α-, β-, and γ-CD appears to cause several changes reflective of an adaptation to a diet containing a poorly digestible carbohydrate. The changes are species dependent, with rats being more susceptible than dogs. In both cases, the effects are reversible upon cessation of treatment.

α-, β-, and γ-CD

The safety of orally administered β-CD has been investigated in numerous studies (120, 144, 166, 167) with extensive evaluation of hematology, blood chemistry, urinalysis, and necropsy (macro and microscopic). No significant toxic effects were observed in any of these studies after oral administration of β-CD to mice, rats, or dogs.

Although no macroscopic pathologies were observed, microscopic evaluation of the tissues revealed several treatment-related changes from the 1-year exposure of rats to β-CD (167). The organs affected by the treatment were the kidneys and the liver. The kidney effects were not thought to be of any toxicological importance.

Some cellular necrosis was observed in the liver of male rats receiving a 5% β-CD diet and in female rats receiving a 2.5 and 5.0% β-CD diet. An increase in portal inflammatory cell infiltration was also observed in male rats receiving the 2.5% β-CD diet and male and female rats receiving the 5.0% β-CD diet. These observations were considered to represent a mild hepatotoxicity (mechanism unknown), which was further evidenced by increases in serum liver enzymes.

The 1-year exposure (167) of dogs to β-CD diets did not result in the kidney or liver pathologies observed in the rats. Therefore, the mild hepatotoxicity may be species related and not reflective of a general hepatotoxicity. Dogs fed 5% β-CD for 1 year exhibited increased urinary protein levels and the urinary excretion of calcium. However, these changes were not noted in the rat study.

From the results of the 1-year studies, the nontoxic effect levels for oral use of β-CD are considered to be 1.25% of the diet for rats and 5% for dogs. Considering the quantity of food that was consumed under these conditions, this is equivalent to approximately 760 and 1899 mg/kg/day for rats and dogs, respectively.

α- and γ-CD show similar oral safety profiles to those observed for β-CD. Ninety-day feeding studies in rats and dogs (153) consuming diets containing α-CD or γ-CD have shown effects that are consistent with the consumption of a poorly digestible carbohydrate such as β-CD or lactose. Some increases in organ weights (spleen and male adrenals) have been observed but were reversible. The ingestion of γ-CD for 13 weeks at dietary levels of up to 20% (corresponding to intakes of 11.4 and 12.7 g/kg body weight/day for male and female rats, respectively) has been shown to be well tolerated (168).

The treatment of dogs with 0, 5, 10, and 20% α-CD and γ-CD diets resulted in minimal effects as compared to those observed in rats (153). A subsequent study concluded that daily γ-CD consumption of up to 20% in the diet (approximately 7.7 g/kg body weight in male and 8.3 g/kg body weight in female dogs) is tolerated without any toxic effects (169).

Table 8 Reported oral safety studies with HP3-β-CD(198) and SBE7-β-CD

Species	Dosing duration (days)	Doses (mg/kg/day)
SBE7-β-CD (Captisol: CyDex)		
Rats	1	600
HP3-β-CD (Encapsin: Janssen)		
Mice	1	5000
	90	500, 2000, 5000
	90	500, 2000, 5000
	730	500, 2000, 5000
Rats	1	5000
	14	5000
	365	500, 2000, 5000
	730	500, 2000, 5000
Dogs	1	5000
	365	500, 1000, 2000

CD derivatives

Oral safety studies have been conducted with at least two derivatives, the HP3-β-CD and SBE7-β-CD. The reported studies for these derivatives are listed in Table 8. The oral safety of HP3-β-CD has been assessed in mice, rats, and dogs for dosing periods up to 2, 2, and 1 year, respectively. Doses reached as high as 5000 mg/kg/day. No adverse effects were noted except for an increase in diarrhea in dogs treated with 5000 mg/kg. The 2-year carcinogenicity studies are discussed separately later.

The oral safety of SBE7-β-CD derivative is currently under evaluation.

Parenteral Safety

The most encompassing test of an excipient's safety is the systemic safety of the material because many of the routes of administration ultimately result in at least some minor systemic exposure. Numerous studies with the parent CDs have shown that their parenteral toxicity is observed primarily as renal and cytotoxicity (hemolysis and tissue

irritation). These toxicities were the driving force for the preparation of new CD derivatives, many of which exhibit improved parenteral safety.

α-, β-, and γ-CD

Renal issues: The parent CDs can all show a toxic effect on the kidney when given parenterally. The nephrotoxicity of α- and β-CD manifests itself as a series of alterations in the organelles of the proximal tubule cells (170). The toxicity is initially expressed as an increase in apical vacuoles, which is typical of an adaptive response to the excretion of osmotic agents at extremely high concentrations. This effect reverses upon discontinuation of CD administration. However, there are also other cellular changes not typical of osmotic agents that are not reversible. Giant lysosomes appear and prominent acicular (needle-like) microcrystals are observed in the epithelial cells of the renal proximal tubules. Both the occurrence and abundance of the microcrystals are dose dependent. The content of the crystals has not been confirmed but suggestions include precipitated parent CD (unlikely for α-CD with a solubility of 145 mg/ml), and complexes of CDs with cholesterol (171) or lipoproteins (172). The proximal tubules progressively show dramatic alterations in other organelles. The mitochondria are observed to swell and become distorted. The Golgi apparatus is affected along with the smooth endoplasmic reticulum. The interstitial membrane on the basal lateral side of the cell is disrupted. All of these events are irreversible, and as the toxic condition progresses, kidney function is lost and death ensues.

Parenterally administered γ-CD appears to be less nephrotoxic than α- or β-CD. Subcutaneous and intravenous doses as high as 4000 mg/kg in mice and 2400 mg/kg in rats have shown no toxic effects (173). Schmid (174) reported that the intravenous LD_{50} for γ-CD in mice was 10,000 mg/kg and >3750 mg/kg for rats. For acute intravenous administration, γ-CD has been shown to be safer than α- and β-CD, which exhibit LD_{50} values of 1000 and 788 mg/kg, respectively, in the rat (170, 174). Antlsperger (153), and more recently Donaubauer (175), evaluated the intravenous administration of γ-CD to rats for 30 and 90 days. A no adverse effect level (NOAEL) of 200 mg/kg was reported for the 30-day studies and 120 mg/kg was suggested for the 90-day studies.

Cytotoxicity issues: In-vivo hemolysis has been observed with parenteral administration of all of the parent CDs. In-vitro studies with human erythrocytes have demonstrated that the damaging effect of the CDs is in the order β-CD > α-CD > γ-CD (176). This cellular destruction has also been observed in studies with human skin fibroblasts and intestinal cells (177), P388 murine leukaemic cells (178), *E. coli* bacterial cells (179), and immortalized human corneal epithelial cells (180). Mechanistic studies suggest that CDs extract either cholesterol (β-CD and γ-CD) or phospholipids (α-CD) from the cell membrane causing small pores which allow leakage and eventually lead to cell lysis.

These in vitro cytotoxicity studies are not indicative of in vivo toxicity but rather provide a method to classify the CDs for their potential to destabilize or disrupt cellular membranes. In fact, when whole blood is used instead of erythrocytes for the hemolysis tests, the cytotoxicity of the CDs is diminished 10-fold by the presence of hydrophobic serum components. Thus, the membrane damaging effects of the CDs are observed in vivo only under situations of high concentrations.

CD derivatives

Renal issues: The derivatized CDs vary widely in their potential for renal safety. Renal nephrosis was observed for methylated β-CDs following intramuscular injections of as little as 50 mg/kg/day over 12 days (181). The damaging effect of the methylated CDs was in the order of TM-β-CD >M-β-CD >DM-β-CD >β-CD. An LD_{50} value of 220 mg/kg has been reported for DM-β-CD (159). Administration of the maltosyl/dimaltosyl derivatives G_2-β-CD/$(G_2)_2$-β-CD on the other hand, showed no toxic effects on the kidney of rats at intravenous doses of 200 mg/kg for 14 days (182). There was however, a flushing of the eyes, nose, mouth and extremities observed, prompting additional investigation.

Safety of the hydroxypropyl and sulfobutyl ether derivatives has been studied in considerable detail and little or no renal toxicity has been demonstrated at moderate doses. Summaries of available intravenous safety studies are given in Table 9. Parenteral exposure with either derivative results in the osmotic adaptive response seen with β-CD but further progression to the irreversible damage does not occur.

Ninety-day intravenous dosing of (2HP)3-β-CD at 400 mg/kg resulted in moderate toxicity as evidenced by decreases in body weight gains, changes in blood and serum parameters, increased activity of mononuclear phagocytosing cells of the lungs and liver, and an increase in the red pulp hyperplasia in the spleen (183).

The evaluation of the sulfobutyl ether derivative, SBE7-β-CD for 6 months with daily intravenous dosing up to 600 mg/kg did not present evidence of the effects noted with (2HP)3-β-CD and demonstrates the extensive systemic safety of this CD.

Cytotoxicity issues: As with renal toxicity, the various derivatives show dramatically different hemolytic behaviors. The dimethyl derivative shows substantial

Table 9 Reported intravenous safety studies with HP3-β-CD(198) and SBE7-β-CD

Species	Dosing duration (days)	Doses (mg/kg/day)
SBE7-β-CD (Captisol: CyDex)		
Mice	1	2000
Rats	1	600
	1	2000
	14	160, 240, 600, 1500, 15000
	30	40, 80, 160
	30	160, 240, 320
	30	300, 1000, 3000
	180	200, 320, 600
Dogs	1	240
	14	160, 240, 750
	30	30, 60, 120
	30	100, 200, 300
	30	300, 1000, 3000
	180	150, 300, 600
HP3-β-CD (Encapsin: Janssen)		
Mice	1	5000, 7000, 10000, 14000, 20000
Rats	1	2000, 4000
	4	1600, 3200
	10	400
	90	25, 50, 100, 400
	90[a]	50, 100, 400
Dogs	1	5000
	4	3200
	90	25, 50, 100, 400
		50, 100, 400

[a]Two 90-day studies were conducted at these levels.
(Adapted from Ref. 198.)

hemolysis; more than even the parent β-CD. This is well illustrated in Fig. 10 where the percentage of cells undergoing hemolysis is shown as a function of CD concentration. Hemolysis started at concentrations below 0.1% for the DM-β-CD. Four to five times higher concentrations of β-CD are required to give the same hemolysis. This behavior is in agreement with the demonstration of DM-β-CD as a penetration enhancer in skin (184) and nasal tissue (185).

The hydroxypropyl and sulfobutyl ether derivatives, on the contrary, show much less hemolysis than β-CD. This is shown in Fig. 11 where the hemolysis caused by β-CD is compared to two (2HP)-β-CDs and three SBE-β-CDs (186). The hemolysis profiles show a dependence on the MS for the derivatives, showing less effect with higher MS. The two hydroxypropyl derivatives, (2HP)3-β-CD and (2HP)7-β-CD, are almost equivalent in their hemolytic behavior and are both less hemolytic than

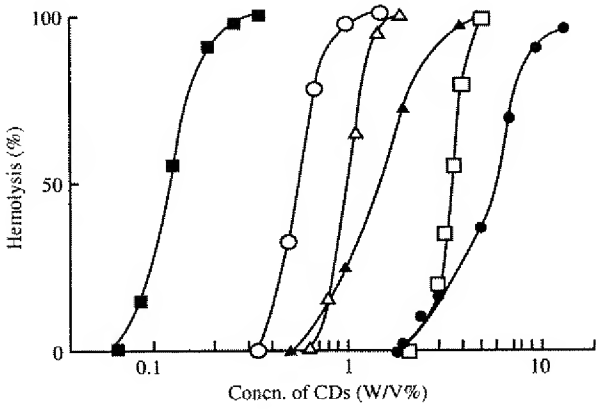

Fig. 10 Hemolytic effects of CD derivatives on human erythrocytes in isotonic phosphate buffer (pH 7.4) at 37 °C for 30 minutes. (Δ, α-CD; ○, β-CD; □, γ-CD; ■, DM-β-CD; ▲, HP-β-CD; ●, HE-β-CD.) (Reprinted from Ref. 135.)

β-CD. Likewise, the sulfobutyl ether derivatives are less hemolytic than β-CD, but the effect of MS is quite dramatic. As the MS increases from one to four to seven, the hemolytic activity drops precipitously to where essentially no hemolysis is observed with SBE7-β-CD.

Mutagenicity and Carcinogenicity

The potential for interaction with genetic material (and therefore risk of carcinogenicity) can be investigated using bacterial and mammalian gene mutation assays and

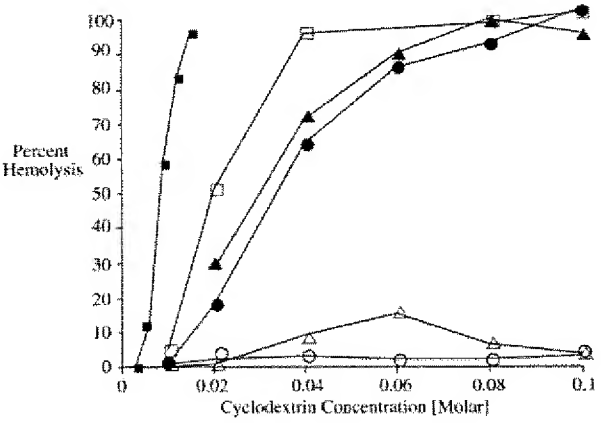

Fig. 11 Hemolytic effects of CD derivatives on human erythrocytes in isotonic phosphate buffer (pH 7.4) at 37 °C for 5 minutes. ■, β-CD; □, SBE1-β-CD; ●, (2HP)3-β-CD;▲, (2HP)7-β-CD; △, SBE4-β-CD; ○, SBE7-β-CD. (Adapted from Ref. 186.)

chromosomal aberration assays. The parent CDs do not exhibit mutagenic behavior in any of these assays (153, 165), and there have been no reports of tumors in oral feeding studies or in the parenteral administration of any of the parent CDs.

Several of the CD derivatives have also been evaluated for mutagenicity and carcinogenicity. Both HP-β-CD (187, 188) and SBE7-β-CD (189) show negative results for the mutagenicity tests. However, a 2-year carcinogenicity study of HP-β-CD in rats demonstrated hyperplastic and neoplastic changes in the acinar cells of the exocrine pancreas (190). The neoplasia in the rat study is inconsistent with the mutagenicity assay results and with the lack of carcinogenicity of the parent CDs. In separate and shorter studies with mice and dogs, no adverse effects were observed for the pancreas.

The rat pancreatic hyperplasia may be due to the ability of high concentration of HP-β-CD to increase the fecal elimination of bile salts indirectly causing a stimulation of the production of cholecystokinin (CCK). In the rats, CCK functions as a mitogen causing an increase in the cellular hyperplasia in the acinar cells. Sensitivity to this effect is species dependent (191), the rat is most sensitive and dog show no effects. The carcinogenicity study for HP-β-CD may have been conducted at levels that are affecting an important nutritional balance. The FDA guidelines for carcinogenicity studies suggest that safety studies be conducted with the highest levels possible to determine maximum tolerated doses but care should be taken to minimize possible nutritional deficiencies (192), The observation of pancreatic neoplasms observed with the 5 g/kg/day oral doses of HP-β-CD may have been the consequence of a nutritional deficiency not a carcinogenic effect of HP-β-CD itself.

Reproductive Safety

In oral-safety studies involving both male and female animals, some minor differences were observed between the sexes. The parent CDs, however, do not adversely affect either gender and the effect of CDs on reproduction is minimal (166). Embryotoxicity and teratogenicity studies have been reported for α- and γ-CD (193). Several 90-day feeding studies in rats and rabbits have been conducted with no effects being observed for maternal health or reproduction (153).

A more extensive evaluation of reproductive and developmental safety of β-CD was reported in a three-generation study by Barrow et al. (194). The only adverse effect observed during the study was a dose related decrease in pup weight gain from birth until weaning but this was statistically significant only for the 5% β-CD diet during days 7–14 postpartum. This preweaning growth retardation did not result in any permanent defects and the affected pups returned to normal weights upon weaning. The NOAEL for oral β-CD, under the conditions of the study, was suggested to be at 1.25% dietary β-CD.

Reproductive safety studies have been conducted for both SBE7-β-CD and HP3-β-CD in rats and rabbits. A listing of the reported studies is given in Table 10. Oral administration of up to 5000 mg/kg HP-β-CD to pregnant rats produced no maternal toxicity, embryotoxicity, or teratogenicity. Oral administration of 1000 mg/kg HP-β-CD to pregnant rabbits caused a slight maternal and embryotoxicity but no teratogenicity.

Intravenous administration of HP-β-CD at 400 mg/kg to the dams from day 18 of the pregnancy to 3 weeks of lactation produced no adverse effects on the rat pups. However, when the dosing occurred from day 16 of gestation to week 3 of lactation, the low dose (50 mg/kg) and the high dose (400 mg/kg) presented significantly lower pup survival than the vehicle control groups.

These effects were not observed with the intravenous administration of SBE7-β-CD at doses of 100, 600, and 3000 mg/kg to pregnant rats. There were no effects of intravenous treatment with SBE7-β-CD on fertility or early embryonic development, nor was the material observed to be teratogenic. The only effect of treatment was a decrease in maternal body weight gains and food consumption at the highest doses administered.

REGULATORY STATUS

Regulatory Process for New Excipients

CDs are not "standard" inactive ingredients, and their uncertain regulatory status causes hesitancy in their use in formulations. A common perception exists that an approval process is in place for the evaluation of new excipients, such as the CDs. In fact, there is no mechanism for submission and review of data on a new excipient that would lead to approval of that excipient. In the United States, the FDA reviews a new excipient only in relationship to the review of a drug formulation. Only the final drug product is approved by the FDA. By this method the excipient data are reviewed with each drug application. The dossier on a new excipient is filed by the excipient manufacturer as a Drug Master File (DMF)-Type 4 (195). These data are then referenced when an Investigational New Drug application (IND) or New Drug Application (NDA) is filed for a drug dosage form using the excipient.

Table 10 Reported reproductive safety studies with HP3-β-CD(198) and SBE7-β-CD

Species	Route	Doses (mg/kg/day)
SBE7-β-CD (Captisol: CyDex)		
Maternal range finding toxicity		
Rats	i.v.	300, 1000, 3000
Rabbits	i.v.	250, 600, 1500
Segment I: Fertility & early embryonic development		
Rats	i.v.	100, 400, 1500
Segment II: Embryotoxicity & teratology		
Rats	i.v.	100, 600, 3000
Rabbits	i.v.	100, 400, 1500
Segment III: Pre- & post-natal development		
Rats	i.v.	100, 600, 3000
HP3-β-CD (Encapsin: Janssen)		
Segment I: Fertility & early embryonic development		
Rats	i.v.	50, 100, 400
	Oral	500, 2000, 5000
Segment II: Embryotoxicity & Teratology		
Rats	i.v.	50, 100, 400
	Oral	500, 2000, 5000
Rabbits	Oral	50, 100, 400
	Oral	250, 500, 1000
Segment III: Pre- & post-natal development		
Rats	i.v.	50, 100, 400
	i.v.	50, 100, 400
	Oral	500, 2000, 5000

(From Ref. 198.)

A petition can also be made for approval as a food additive and to be placed on the GRAS (generally regarded as safe) list. The GRAS list (21 Code of Federal Regulations 182.1–184.1) actually applies only to food additives that are reviewed by the FDA and determined to be generally recognized as safe for the purpose and use conditions described in the statute. The use of GRAS excipients is often but not always transferable to oral pharmaceutical formulations. Once the material is approved for use in foods, the material may be considered suitable for use in an oral formulation if the dose fits within the quantities consumed as a food additive. This suitability does not, however, transfer to non-oral routes.

The process is similar in Japan, in that a new excipient's dossier is evaluated in reference to an application for a drug dosage form containing the excipient. The data is evaluated both in terms of the excipient and the active, but only the drug product is approved. After the excipient has seen extensive utilization in multiple marketed products, the regulatory system has a process for review of the data resulting in possible inclusion of the monograph in the *Japanese Pharmacopoeia* (JP). The JP defines the mandatory standards for substances used in a pharmaceutical product. Inclusion in the JP establishes "precedent" status for the excipient and this notation permits use of the material in new drug products under defined conditions without the need to submit extensive supporting data.

In Japan however, new is new. Even with precedent status, if a new higher dose or a new route of administration is pursued, the examination will treat the excipient as new. This also applies to an approved food additive or cosmetic ingredient. The first use in a pharmaceutical formulation is considered a new use and the application will be examined as such.

Current Regulatory Status of CDs

The parent CDs in Japan are classified as natural starches that have received approval by the Ministries of Health for use in foods. Relative to pharmaceutical applications,

monographs for α- and β-CD have been included in the Japanese Pharmaceutical Excipients (196) compendium (JPE). Even though nine pharmaceutical products with CD formulations have been marketed in Japan, the use of CDs has not been extensive enough in approved formulations to receive "precedent status."

In the United States, two drug products are approved containing CDs (one each containing α-CD and (2HP)-β-CD) and at least one additional NDA has been filed (product containing SBE7-β-CD). In addition, Drug Master Files have been submitted for β- and γ-CD and the (2HP)-β-CD and SBE7-7β-CD derivatives. These DMFs are available for referencing in IND and NDA applications through agreements with the individual manufacturers. A food additive petition is also under review for β-CD, and a monograph on β-CD has been included in volume 19 of the NF under the name Betadex (197). The United States Pharmacopeal Convention is reviewing proposal(s) to include monographs on additional derivative(s).

An expert panel concluded in 1997 that β-CD is GRAS for its intended use as a flavor carrier and protectant at a level of 2% in numerous food products. The products included chewing gum, gleatin and puddings, soups prepared from dry mixes, coffee and tea products with added flavors, savory snacks and crackers with added flavorings, baked goods prepared from dry mixes, beverages prepared from dry mixes, and breakfast cereals. A petition has been filed with the FDA requesting their affirmation of β-CD as GRAS for these products. The Joint Expert Committee on Food Additives (JECFA) of the World Health Organization and the Food and Agriculture Organization has reviewed β-CD and established an acceptable daily intake (ADI) of 0–5 mg/kg body weight. The Scientific Committee for Foods of the European Union has also assigned β-CD an ADI of 5 mg/kg body weight/day. The FDA granted GRAS status for γ-CD in 2000.

REFERENCES

1. Villiers, A. Sur La Fermentation De La Fécule Par L'action Du Ferment Butyrique. C R Hebd. Seances Acad. Sci. **1891**, *112*, 536–538.
2. Schardinger, F. Thermophile Bakterien Aus Verschiedenen Nahrungsmittein Und Milch Und Die Gebildeten Produkte, Wenn Diese Bakterien in Nahrlosungen Kultivert Werden, Die Kohlenhydrate Enthalen. Z. Unters. Nahr. Genussm. **1903**, *6*, 865.
3. French, D.; Rundle, R.E. The Molecular Weights of the Schardinger Alpha and Beta Dextrins. J. Am. Chem. Soc. **1942**, *64*, 1651–1653.
4. Freudenberger, K.; Cramer, F. Die Konstitution Der Schardinger Dextrine Alpha, Beta, Und Gamma. Z. Naturforsch. **1948**, *3b*, 464.
5. Procedings of 10th International Symposium on Cyclodextrins Ann Arbor MI May 2000, Schmid, G., Eds.; MIRA Digital Publishing: St. Louis, MO, 2001.
6. Thompson, D.O. Cyclodextrins-Enabling Excipients: their Present and Future Use in Pharmaceuticals. Crit. Rev. Ther. Drug Carrier Syst. **1997**, *14* (1), 1–104.
7. Frömming, K.-H.; Szejtli, J. Cyclodextrins in Pharmacy. Kluwer Academic Publishers: Dordrecht, The Netherlands, 1994; 224.
8. Uekama, K.; Otagiri, M. Cyclodextrins in Drug Carrier Systems. CRC Crit. Rev. Ther. Drug Carrier Syst. **1987**, *3* (1), 1–40.
9. Higuchi, T.; Connors, K.A. Phase-Solubility Techniques. *Advances in Analytical Chemistry and Instrumentation*, Reilly, C.N., Ed.; John Wiley & Sons, Inc.: New York, 1965; 4, 117–212.
10. Merino, C.; Junquera, E.; Jimenez-Barbero, J.; Aicart, E. Effect of the Presence of β-Cyclodextrin on the Solution Behavior of Procaine Hydrochloride. Spectroscopic and Thermodynamic Studies. Langmuir **2000**, *16* (4), 1557–1565.
11. Maupas, B.; Letellier, S.; Guyon, F. Determination of the Formation Constant for the Inclusion Complex of Methyl-β-Cyclodextrin with Anticoagulant Drugs Warfarin and 8-Chlorowarfarin in Aqueous Solution. J. Inclusion Phenom. Mol. Recognit. Chem. **1996**, *23* (4), 259–267.
12. Sadlej-Sosnowska, N. Fluorometric Determination of Association Constants of Three Estrogens with Cyclodextrins. J. Fluoresc. **1997**, *7* (3), 195–200.
13. Escandar, G.M. Spectrofluorimetric Determination of Piroxicam in the Presence and Absence of β-Cyclodextrin. Analyst **1999**, *124* (4), 587–591.
14. Nishijo, J.; Ushiroda, Y. Interaction of 2-Naphthalenesulfonate With β-Cyclodextrin: Studies with Calorimetry and Proton Nuclear Magnetic Resonance Spectroscopy. Chem. Pharm. Bull. **1998**, *46* (11), 1790–1796.
15. Li, S.; Purdy, W.C. Circular Dichroism, Ultraviolet, and Proton Nuclear Magnetic Resonance Spectroscopic Studies of the Chiral Recognition Mechanism of β-Cyclodextrin. Anal. Chem. **1992**, *64* (13), 1405–1412.
16. Patonay, G.; Warner, I.M. Investigation of Induced Circular Dichroism of Benzo(a)pyrene Cyclodextrin Complexes. J. Inclusion Phenom. Mol. Recognit. Chem. **1991**, *11* (4), 313–322.
17. DePonti, R.; Torricelli, C.; Motta, A.; Crivellente, M. Use of a Polarographic Method, a UV Method, and the Phase-Solubility Technique to Determine the Stability Constant in Aqueous Solution of a β-Cyclodextrin Complex with a New Immunomodulating Agent. Eur. J. Pharm. Biopharm. **1991**, *37* (2), 106–109.
18. Koenigbauer, M.J. Pharmaceutical Applications of Microcalorimetry. Pharm. Res. **1994**, *11* (6), 777–783.
19. Mwakibete, H.; Cristantino, R.; Bloor, D.M.; Wyn-Jones, E.; Holzwarth, J.F. Reliability of the Experimental Methods to Determine Equilibrium Constants for Surfactant/zCyclodextrin Inclusion Complexes. Langmuir **1995**, *11* (1), 57–60.
20. Dharmawardana, U.R.; Christian, S.D.; Tucker, E.E.; Taylor, R.W.; Scamehorn, J.F. A Surface Tension Method

for Determining Binding Constants for Cyclodextrin Inclusion Complexes of Ionic Surfactants. Langmuir **1993**, *9* (9), 2258–2263.
21. Ono, N.; Hirayama, F.; Arima, H.; Uekama, K. Determination of Stability Constant of β-Cyclodextrin Complexes Using the Membrane Permeation Technique and the Permeation Behavior of Drug-Competing Agent-β-Cyclodextrin Ternary Systems. Eur. J. Pharm. Sci. **1999**, *8* (2), 133–139.
22. Bartak, P.; Bednar, P.; Kubacek, L.; Stransky, Z. Advanced Statistical Evaluation of Complex Formation Constant from Electrophoretic Data. Anal. Chim. Acta **2000**, *407* (1/2), 327–336.
23. Neubert, R.H.H.; Schwarz, M.A.; Mrestani, Y.; Platzer, M.; Raith, K. Affinity Capillary Electrophoresis in Pharmaceutics. Pharm. Res. **1999**, *16* (11), 1663–1673.
24. Suzuki, M.; Ito, K.; Fushimi, C.; Kondo, T. Application of Freezing Point Depression to Drug Interaction Studies. I. a Study of 1:1 Plus 1:2 Complexes Between Barbiturate and α-Cyclodextrin Using the Freezing Point Depression Method. Chem. Pharm. Bull. **1993**, *41* (8), 1444–1447.
25. Thuaud, N.; Sebille, B.; Deratani, A.; Lelievre, G. Determination by High-Performance Liquid Chromatography of the Binding Properties of Charged β-Cyclodextrin Derivatives with Drugs. J. Chromatogr. **1990**, *503* (2), 453–458.
26. Hamai, S.; Handa, M. Paper Chromatographic Study on the Inclusion Complexes of Cyclodextrins and Methyl Orange. Anal. Lett. **1999**, *32* (5), 1037–1047.
27. Wang, M.; Ueda, H.; Nagai, T. Simple Prediction of Stability Constants for Inclusion Complexes of β-Cyclodextrin with Various Drug Molecules Using β-Cyclodextrin Bonded Phases (Cyclobond I Column). Drug Dev. Ind. Pharm. **1990**, *16* (4), 571–579.
28. Cserhati, T.; Forgacs, E. Charge-Transfer Chromatographic Study of the Complex Formation of Some Steroidal Drugs with Carboxymethyl γ-Cyclodextrin. Anal. Biochem. **1997**, *246* (2), 205–210.
29. Lederer, M.; Leipzig-Pagani, E. A Simple Alternative Determination of the Formation Constant for the Inclusion Complex Between Rutin and β-Cyclodextrin. Anal. Chim. Acta **1996**, *329* (3), 311–314.
30. Kraus, C.; Mehnert, W.; Froemming, K.H. Interactions of β-Cyclodextrin With Solutol HS 15 and their Influence on Diazepam Solubilization. PZ Wiss. **1991**, *4* (1), 11–15.
31. Lin, S.Y.; Kao, Y.H.; Yang, J.C. Grinding Effect on Some Pharmaceutical Properties of Drugs by Adding β-Cyclodextrin. Drug Dev. Ind. Pharm. **1988**, *14* (1), 99–118.
32. Abdel-Rahman, S.I.; El-Sayed, A.M. Interaction of Some Benzothiadiazine Diuretics with β-Cyclodextrin. Acta Pharm. Hung. **1990**, *60* (2/3), 69–75.
33. Bekers, O.; Beijnen, J.H.; Otagiri, M.; Bult, A.; Underberg, W.J.M. Inclusion Complexation of Doxorubicin and Daunorubicin with Cyclodextrins. J. Pharm. Biomed. Anal. **1990**, *8* (12), 671–674.
34. Husain, N.; Ndou, T.T.; Munoz de la Pena, A.; Warner, I.M. Complexation of Doxorubicin with β- and γ-Cyclodextrins. Appl. Spectrosc. **1992**, *46* (4), 652–658.
35. Imai, T.; Irie, T.; Otagiri, M.; Uekama, K.; Yamasaki, M. Comparative Study on Inclusion Complexations of Antiinflammatory Drug Flurbiprofen with β-Cyclodextrin and Methylated Beta-Cyclodextrins. J. Inclusion Phenom. **1984**, *2* (3/4), 597–604.
36. Abdel-Rahman, A.A.; Saleh, S.I.; Nakai, Y.; Aboutaleb, A.E.; Ahmed, M.O. Investigation of the Interaction of Bromazepam with Cyclodextrins in Solutions and in Ground Mixtures. J. Pharm. Belg. **1994**, *49* (1), 23–32.
37. Ahmed, M.O.; Nakai, Y.; Aboutaleb, A.E.S.; Yamamoto, K.; Abdel Rahman, A.A.Z.; Saleh, S.I. Complex Formation of Nitrazepam in Coprecipitating and in Co-grinding with Methylated Beta-Cyclodextrins. Chem. Pharm. Bull. **1990**, *38* (12), 2423–2427.
38. Müller, B.W.; Brauns, U. Solubilization of Drugs by Modified Beta-Cyclodextrins. Int. J. Pharm. **1985**, *26* (1/2), 77–88.
39. Müller, B.W.; Brauns, U. Hydroxypropyl-Beta-Cyclodextrins Derivatives: Influence of Average Degree of Substitution on Complexing Ability and Surface Activity. J. Pharm. Sci. **1986**, *75* (6), 571–572.
40. Yoshida, A.; Yamamoto, M.; Irie, T.; Hirayama, F.; Uekama, K. Some Pharmaceutical Properties of 3-Hydroxypropyl- and 2,3-Dihydroxypropyl-β-Cyclodextrins and their Solubilizing and Stabilizing Abilities. Chem. Pharm. Bull. **1989**, *37* (4), 1059–1063.
41. Rao, C.T.; Pitha, J.; Lindberg, B.; Lindberg, J. Distribution of Substituents In *O*-(2-Hydroxypropyl) Derivatives of Cyclomalto-Oligosaccharides (Cyclodextrins): Influence of Increasing Substitution, of the Base Used in the Preparation, and of Macrocyclic Size. Carbohydr. Res. **1992**, *223*, 99–107.
42. Kano, K.; Zhou, B.; Sakaguchi, M.; Matsumoto, H.; Hashimoto, S. Effects of Aeration, Freezing and Thawing on Self-Aggregation of Naphthalene in Aqueous β-Cyclodextrin Solutions. Sci. Eng. Rev. Doshisha Univ. **1985**, *25* (4), 253–259.
43. Menger, F.M.; Williams, D.Y. Synthesis and Properties of a Surfactant-Cyclodextrin Conjugate. Tetrahedron Lett. **1986**, *27* (23), 2579–2582.
44. Stella, V.; Rajewski, R.A. Derivatives of Cyclodextrins Exhibiting Enhanced Aqueous Solubility and the Use Thereof. US Patent 5,134,127, 1992.
45. Gerloczy, A.; Hoshino, T.; Pitha, J. Safety of Oral Cyclodextrins: Effects of (Hydroxypropyl) Cyclodextrins, Cyclodextrin Sulfates and Cationic Cyclodextrins on Steroid Balance in Rats. J. Pharm. Sci. **1994**, *83* (2), 193–196.
46. Okimoto, K.; Rajewski, R.A.; Jona, J.A.; Stella, V.J. The Interaction of Charged and Uncharged Drugs with a Neutral (HP-β-Cyclodextrin) and Anionically Charged (SBE7-b-Cyclodextrin) β-Cyclodextrin. Pharm. Res. **1996**, *13* (2), 256–264.
47. Badawy, S.I.F.; Marshall, A.L.; Ghorab, M.M.; Adeyeye, C.M. A Study of the Complexation Between Danazol and Hydrophilic Cyclodextrins Derivatives. Drug Devel. Indust. Pharm. **1996**, *22* (9/10), 959–966.
48. Rajewski, R.A. Ph.D. Dissertation. *Development and Evaluation of the Usefulness and Parenteral Safety of Modified Cyclodextrins*; University of Kansas: Lawrence, KS USA, 1991.
49. Johnson, M.D.; Hoesterey, B.L.; Anderson, B.D. Solubilization of a Tripeptide HIV Protease Inhibitor Using a Combination of Ionization and Complexation with

Chemically Modified Cyclodextrins. J. Pharm. Sci. **1994**, *83* (8), 1142–1146.
50. Rajewski, R.A.; Traiger, G.; Bresnahan, J.; Jaberaboansari, P.; Stella, V.J.; Thompson, D.O. Preliminary Safety Evaluation of Parenterally Administered Sulfoalkyl Ether β-Cyclodextrin Derivatives. J. Pharm. Sci. **1995**, *84* (8), 927–932.
51. Otero-Espinar, F.J.; Anguiano-Igea, S.; Garcia-Gonzalez, N.; Vila-Jato, J.L.; Blanco-Mendez, J. Interaction of Naproxen with β-Cyclodextrin in Solution and in the Solid State. Int. J. Pharm. **1992**, *79* (2/3), 149–157.
52. Hoshino, T.; Uekama, K.; Pitha, J. Increase in Temperature Enhances Solubility of Drugs in Aqueous Solutions of Hydroxypropyl Cyclodextrins. Int. J. Pharm. **1993**, *98* (1/3), 239–242.
53. Menard, F.A.; Dedhiya, M.G.; Rhodes, C.T. Physicochemical Aspects of the Complexation of Some Drugs with Cyclodextrins. Drug Dev. Ind. Pharm. **1990**, *16* (1), 91–113.
54. Pitha, J.; Hoshino, T. Effects of Ethanol on Formation of Inclusion Complexes of Hydroxypropyl Cyclodextrins with Testosterone or with Methyl Orange. Int. J. Pharm. **1992**, *80* (2/3), 243–251.
55. Reer, O.; Müller, B.W. The Influence of Cosolvents and Surfactants on the Complexation of Dexamethasone with Hydroxypropyl-β-Cyclodextrin Investigated with a Simplex Lattice Design. Eur. J. Pharm. Biopharm. **1993**, *39* (3), 105–111.
56. Mulski, M.J.; Connors, K.A. Solvent Effects on Chemical Processes. 9. Energetic Contributions to the Complexation of 4-Nitroaniline with Alpha-Cyclodextrin in Water and in Binary Aqueous-Organic Solvents. Supramol. Chem. **1995**, *4* (4), 271–278.
57. Loftsson, T.; Masson, M.; Sigurjonsdottir, J.F. Methods to Enhance the Complexation Efficiency of Cyclodextrins. Stp Pharma Sciences **1999**, *9* (3), 237–242.
58. Redenti, E.; Szente, L.; Szejtli, J. Drug/Cyclodextrin/Hydroxy Acid Multicomponent Systems. Properties and Pharmaceutical Applications. J. Pharm. Sci. **2000**, *89* (1), 1–8.
59. Turro, N.J.; Okubo, T.; Chung, C.J. Analysis of Static and Dynamic Host-Guest Associations of Detergents with Cyclodextrins via Photoluminescence Methods. J. Am. Chem. Soc. **1982**, *104* (7), 1789–1794.
60. Hashimoto, S.; Thomas, J.K. Fluorescence Study of Pyrene and Naphthalene in Cyclodextrin-Amphiphile Complex Systems. J. Am. Chem. Soc. **1985**, *107* (16), 4655–4662.
61. Stella, V.J.; Rao, V.M.; Zannou, E.A.; Zia, V. Mechanisms of Drug Release from Cyclodextrin Complexes. Adv. Drug Del. Rev. **1999**, *36* (1), 3–16.
62. Szejtli, J. Medicinal Applications of Cyclodextrins. Med. Res. Rev. **1994**, *14* (3), 353–386.
63. Hirai, S. Formulation Studies of Cefotiam Hexetil Hydrochloride: Effect of α-Cyclodextrin as Dissolution Enhancer. *Proceedings of 7th International Symposium on Cyclodextrins*; Osa, T., Ed.; Komiyama Printing Co., Ltd.: Tokyo, 1994; 39–44.
64. Järvinen, T.; Järvinen, K.; Schwarting, N.; Stella, V.J. β-Cyclodextrin Derivatives, SBE4-β-Cyclodextrin and HP-β-Cyclodextrin, Increase the Oral Bioavailability of Cinnarizine in Beagle Dogs. J. Pharm. Sci. **1995**, *84* (3), 295–299.
65. Soliman, O.A.; Kimura, K.; Hirayama, F.; Uekama, K.; El-Sabbagh, H.M.; El-Gawad, A.E.; Hashim, F.M. Amorphous Spironolactone-Hydroxypropylated Cyclodextrin Complexes with Superior Dissolution and Oral Bioavailability. Int. J. Pharm. **1997**, *149* (1), 73–83.
66. Saket, M. Improvement of Solubility and Dissolution Rate of Meclizine Hydrochloride Utilizing Cyclodextrins and Non-Ionic Surfactant Solutions Containing Cosolvents and Additives. Acta Technologiae et Legis Medicamenti **1997**, *8* (1), 33–48.
67. Nagarsenker, M.S.; Tantry, J.S.; Shenai, H. Influence of Hydroxypropyl-β-Cyclodextrin on the Dissolution of Ketoprofen and Irritation to Gastric Mucosa after Oral Administration in Rats. Pharm. Sci. **1997**, *3* (9), 443–445.
68. Moyano, J.R.; Arias, M.J.; Gines, J.M.; Perez, J.I.; Rabasco, A.M. Dissolution Behavior of Oxazepam in Presence of Cyclodextrins: Evaluation of Oxazepam-DIMEB Binary System. Drug Dev. Ind. Pharm. **1997**, *23* (4), 379–385.
69. Badawy, S.I.F.; Ghorab, M.M.; Adeyeye, C. Study of the Complexation Between Danazol and Hydrophilic Cyclodextrin Derivatives. Pharm. Res. **1995**, *12* (9), S204.
70. Savolainen, J.; Jarvinen, K.; Matilainen, L.; Jarvinen, T. Improved Dissolution and Bioavailability of Phenytoin by Sulfobutylether-β-Cyclodextrin ((Sbe)(7m)-β-Cd) and Hydroxypropyl-B-Cyclodextrin (Hp-β-Cd) Complexation. Int. J. Pharm. **1998**, *165* (1), 69–78.
71. Kimura, K.; Hirayama, F.; Arima, H.; Uekama, K. Effects of Aging on Crystallization, Dissolution and Absorption Characteristics of Amorphous Tolbutamide-2-Hydroxypropyl-β-Cyclodextrin Complex. Chem. Pharm. Bull. **2000**, *48* (5), 646–650.
72. Vora, J.; Boroujerdi, M. Enhanced Aqueous Solubility of Phenolic Antioxidants Using Modified Beta-Cyclodextrins. Drug Dev. Ind. Pharm. **1995**, *21* (4), 495–502.
73. Lehner, S.J.; Müller, B.W.; Seydel, J.K. Effect of Hydroxypropyl-β-Cyclodextrin on the Antimicrobial Action of Preservatives. J. Pharm. Pharmacol. **1994**, *46* (3), 186–191.
74. Loftsson, T.; Stefansdottir, O.; Frioriksdottir, H.; Guomundsson, O. Interactions Between Preservatives and 2-Hydroxypropyl β-Cyclodextrin. Drug Dev. Ind. Pharm. **1992**, *18* (13), 1477–1484.
75. Nunuz-Delicado, E.; Sanchez-Ferrer, A.; Garcia-Carmona, F. Cyclodextrins As Secondary Antioxidants: Synergism With Ascorbic Acid. J. Agric. Food Chem. **1997**, *45* (8), 2830–2835.
76. Otero Espinar, F.J.; Anguiano Igea, S.; Blanco Mendez, J.; Vila Jato, J.L. Reduction in the Ulcerogenicity of Naproxen by Complexation with β-Cyclodextrin. Int. J. Pharm. **1991**, *70* (1/2), 35–41.
77. Park, J.H.; Kim, J.H.; Kim, J.I.; Kim, S.J.; Seo, S.H.; Lee, K. Comparison of Diclofenac Sodium and Diclofenac Sodium-β-Cyclodextrin Complexation on Gastric Mucosal Injury in Rats. Yakche Hakhoechi **1997**, *27* (1), 11–14.
78. Santucci, L.; Fiorucci, S.; Chiucchiu, S.; Sicilia, A.; Bufalino, L.; Morelli, A. Placebo-Controlled Comparison of Piroxicam-β-Cyclodextrin, Piroxicam, and Indomethacin on Gastric Potential Difference and Mucosal Injury in Humans. Dig. Dis. Sci. **1992**, *37* (12), 1825–1832.

79. Loftsson, T.; Jarvinen, T. Cyclodextrins in Ophthalmic Drug Delivery. Adv. Drug Del. Rev. **1999**, *36* (1), 59–79.
80. Doenicke, A.; Roizen, M.F.; Nebauer, A.E.; Kugler, A.; Hoernecke, R.; Beger-Hintzen, H. A Comparison of Two Formulations for Etomidate, 2-Hydroxypropyl-β-Cyclodextrin (HPCyclodextrin) and Propylene Glycol. Anesth. Analg. (NY) **1994**, *79* (5), 933–939.
81. Stella, V.J.; Lee, H.K.; Thompson, D.O. The Effect of SBE4-b-Cyclodextrin on I.m. Prednisolone Pharmacokinetics and Tissue Damage in Rabbits: Comparison to a Co-Solvent Solution and a Water-Soluble Prodrug. Int. J. Pharm. **1995**, *120* (2), 197–204.
82. Howard, D.; Morris, R.M.; Rinkema, L.; Morrow, T.E.; Laska, D.A. Moderation of Untoward Side Effects during In Vivo and In Vitro Screening of Potential I.Vz. Preparations. *Min. 6th Int. Symp. Cyclodextrins*; Hedges, A.R., Ed.; Editions de Santé: Paris, 1992; 474–477.
83. Weiszfeiler, V.; Szejtli, J. Bitterness Reduction with β-Cyclodextrin. *Proceedings of 4th International Symposium of Cyclodextrins*; Huber, O.; Szejtli, J., Eds.; Kluwer Academic Publishers: Dordrecht The Netherlands, 1988; 359–366.
84. Funasaki, N.; Kawaguchi, R.; Ishikawa, S.; Hada, S.; Neya, S.; Katsu, T. Quantitative Estimation of the Bitter Taste Intensity of Oxyphenonium Bromide Reduced by Cyclodextrins from Electromotive Force Measurements. Anal. Chem. **1999**, *71* (9), 1733–1736.
85. Funasaki, N.; Uemura, Y.; Hada, S.; Neya, S. Reduction of the Bitter Taste Intensity of Propantheline Bromide by Cyclodextrins as Predicted by Surface Tension Measurements. J. Phys. Chem **1996**, *100* (40), 16298–16301.
86. Uekama, K.; Oh, K.; Otagiri, M.; Seo, H.; Tsuruoka, M. Improvement of Some Pharmaceutical Properties of Clofibrate by Cyclodextrin Complexation. Pharm. Acta Helv. **1983**, *58* (12), 338–342.
87. Loftsson, T. Effects of Cyclodextrins on the Chemical Stability of Drugs in Aqueous Solutions. Drug Stab. **1995**, *1* (1), 22–33.
88. Loftsson, T.; Brewster, M.E. Pharmaceutical Applications of Cyclodextrins. 1. Drug Solubilization and Stabilization. J. Pharm. Sci. **1996**, *85* (10), 1017–1025.
89. Ueda, H.; Ou, D.; Endo, T.; Nagase, H.; Nagai, T. Evaluation of a Sulfobutyl Ether β-Cyclodextrin as a Solubilizing/Stabilizing Agent for Several Drugs. *Symposium on Pharmaceutical Applications of Cyclodextrins*; Lawrence, KS, June 29 – July 2, 1997.
90. Ma, D.Q.; Rajewski, R.A.; Vander Velde, D.; Stella, V.J. Comparative Effects of (Sbe)(7m)-β-Cd and Hp-β-Cd on the Stability of Two Anti-Neoplastic Agents, Melphalan and Carmustine. J. Pharm. Sci. **2000**, *89* (2), 275–287.
91. Schlenk, H.; Sand, D.M.; Tillotson, J.A. Stabilization of Autoxidizable Materials by Means of Inclusion. J. Am. Chem. Soc. **1955**, *77*, 3587–3590.
92. Hirayama, F.; Kurihara, M.; Uekama, K. Improving the Aqueous Stability of Prostaglandin E2 and Prostaglandin A2 by Inclusion Complexation With Methylated-β-Cyclodextrins. Chem. Pharm. Bull. **1984**, *32* (10), 4237–4240.
93. Jarho, P.; Vander Velde, D.; Stella, V.J. Cyclodextrin-Catalyzed Deacetylation of Spironolactone is pH And Cyclodextrin Dependent. J. Pharm. Sci. **2000**, *89* (2), 241–249.
94. Oguchi, T.; Yonemochi, E.; Yamamoto, K.; Nakai, Y. Freeze-Drying of Drug-Additive Binary Systems. II. Relationship Between Decarboxylation Behavior and Molecular States of p-Aminosalicylic Acid. Chem. Pharm. Bull. **1989**, *37* (11), 3088–3091.
95. Mielcarek, J. Photochemical Stability of the Inclusion Complexes of Nicardipine with α-, γ-Cyclodextrin, Methyl-β-Cyclodextrin, and Hydroxypropyl-β-Cyclodextrin in the Solid State and in Solution. Pharmazie **1996**, *51* (7), 477–479.
96. Ammar, H.O.; El-Nahhas, S.A. Improvement of Some Pharmaceutical Properties of Drugs by Cyclodextrin Complexation. 2. Colchicine. Pharmazie **1995**, *50* (4), 269–272.
97. Yamamoto, M.; Hirayama, F.; Uekama, K. Improvement of Stability and Dissolution of Prostaglandin E1 by Maltosyl Beta-Cyclodextrin in Lyophilized Formulation. Chem. Pharm. Bull. **1992**, *40* (3), 747–751.
98. Cwiertnia, B.; Hladon, T.; Stobiecki, M. Stability of Diclofenac Sodium in the Inclusion Complex with β-Cyclodextrin in the Solid State. J. Pharm. Pharmacol. **1999**, *51* (11), 1213–1218.
99. Lee, H.J.; Kim, S.U.; Seo, S.H. Pharmaceutical Studies on the Drug (III). Enhancement of Dissolution and Stability of Sulfamethoxazole by Beta-Cyclodextrin Complexation. Yakche Hakhoechi **1988**, *18* (4), 181–186.
100. Sigurjonsdottir, J.F.; Loftsson, T.; Masson, M. Influence of Cyclodextrins on the Stability of the Peptide Salmon Calcitonin in Aqueous Solution. Int. J. Pharm. **1999**, *186* (2), 205–213.
101. Sah, H. Stabilization of Proteins Against Methylene Chloride Water Interface-Induced Denaturation and Aggregation. J. Controlled Release **1999**, *58* (2), 143–151.
102. Karuppiah, N.; Sharma, A. Cyclodextrins As Protein Folding Aids. Biochem. Biophys. Res. Commun. **1995**, *211* (1), 60–66.
103. Tokihiro, K.; Irie, T.; Uekama, K. Varying Effects of Cyclodextrin Derivatives on Aggregation and Thermal Behavior of Insulin in Aqueous Solution. Chem. Pharm. Bull. **1997**, *45* (3), 525–531.
104. Katakam, M.; Banga, A.K. Aggregation of Proteins and Its Prevention by Carbohydrate Excipients: Albumins and γ-Globulin. J. Pharm. Pharmacol. **1995**, *47* (2), 103–107.
105. Cooper, A.; Lovatt, M.; Nutley, M.A. Energetics of Protein-Cyclodextrin Interactions. J. Inclusion Phenom. Mol. Recognit. Chem. **1996**, *25* (1–3), 85–88.
106. Sharma, A. Cyclodextrin-Assisted Protein Folding. *Symposium on Pharmaceutical Applications of Cyclodextrins*; Lawrence, KS, June 29 - July 2, 1997.
107. Utsuki, T.; Hirayama, F.; Uekama, K. Different Photodimerization Behavior of Tranilast in α-, β- and Gamma-Cyclodextrin Complexes: Cavity-Size and Stoichiometry Dependence. J. Chem. Soc., Perkin Trans **1993**, *2* (1), 109–114.
108. Lin, G.; Xu, S. A New Method of Encapsulating Volatile Oils in Chinese Medicinal Herbs with Beta-Cyclodextrin. Zhongguo Zhongyao Zazhi **1992**, *17* (5), 283–285.
109. Qi, Z.H.; Hedges, A.R. Use of Cyclodextrins for Flavors. ACS Symp. Ser., Flavor Technology **1995**, *610*, 231–243.
110. Bhandari, B.R.; D'arcy, B.R.; Bich, L.L.T. Lemon Oil to β-Cyclodextrin Ratio Effect on the Inclusion Efficiency of

β-Cyclodextrin and the Retention of Oil Volatiles in the Complex. J. Agric. Food Chem. **1998**, *46* (4), 1494–1499.
111. Uekama, K.; Oh, K.; Irie, T.; Otagiri, M.; Nishimiya, Y.; Nara, T. Stabilization of Isosorbide 5-Mononitrate in Solid State by β-Cyclodextrin Complexation. Int. J. Pharm. **1985**, *25* (3), 339–346.
112. Umemura, M.; Ueda, H.; Tomono, K.; Nagai, T. Effect of Diethyl Beta-Cyclodextrin on the Release of Nitroglycerin from Formulations. Drug Des. Delivery **1990**, *6* (4), 297–310.
113. Szente, L.; Apostol, I.; Szejtli, J. Suppositories Containing Beta-Cyclodextrin Complexes. Part 1: Stability Studies. Pharmazie **1984**, *39* (10), 697–699.
114. Saenger, W.; Betzel, C.; Hingerty, B.; Brown, G.M. Flip-Flop Hydrogen Bridging Bonds in Beta-Cyclodextrin—A General Principle in Polysaccharides. Angew. Chem. **1983**, *95* (11), 883–884.
115. Jozwiakowski, M.J.; Connors, K.A. Aqueous Solubility Behavior of Three Cyclodextrins. Carb. Res. **1985**, *143*, 51–59.
116. Coleman, A.W.; Nicolis, I.; Keller, N.; Dalbiez, J.P. Aggregation of Cyclodextrins: An Explanation of the Abnormal Solubility of Beta-Cyclodextrin. J. Inclusion Phenom. Mol. Recognit. Chem. **1992**, *13* (2), 139–143.
117. Pedersen, M. Effect of Hydrotropic Substances on the Complexation of Clotrimazole with Beta-Cyclodextrin. Drug Dev. Ind. Pharm. **1993**, *19* (4), 439–448.
118. Coleman, A.W.; Nicolis, I. Inorganic Salt Modulation of the Aqueous Solubility of Beta-Cyclodextrin. Supramol. Chem. **1993**, *2* (2/3), 93–97.
119. Loftsson, T.; Frioriksdottir, H. The Effect of Water-Soluble Polymers on the Aqueous Solubility and Complexing Abilities of β-Cyclodextrin. Int. J. Pharm. **1998**, *163* (1/2), 115–121.
120. Szejtli, J. Cyclodextrins and their Inclusion Complexes. *Akadémiai Kiadó*: Budapest Hungary, 1982; 296.
121. Schonberger, B.P.; Jansen, A.C.A.; Janssen, L.H.M. The Acid Hydrolysis of Cyclodextrins and Linear Oligosaccharides: A Comparative Study. *Proceedings of 4th International Symposium on Cyclodextrins*; Huber, O.; Szejtli, J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988; 61–63.
122. Szejtli, J.; Budai, Z. Acid Hydrolysis of Beta-Cyclodextrin. Acta Chim. Acad. Sci. Hung. **1976**, *91* (1), 73–80.
123. Al-Rawi, A.M. The Susceptibility of Gamma-Cyclodextrin (Schardinger Dextrin) to Ionizing Radiation. *Food Preserv. Irradiat., Proceedings of the International Symposium*; IAEA: Vienna Austria, 1978; 487–500.
124. Nakai, Y.; Yamamoto, K.; Terada, K.; Kajiyama, A.; Sasaki, I. Properties of Crystal Water of α-, β-, and Gamma;-Cyclodextrin. Chem. Pharm. Bull. **1986**, *34* (5), 2178–2182.
125. Lindner, K.; Saenger, W. Topography of Cyclodextrin Complexes. Part XVII. Crystal and Molecular Structure of Cycloheptaamylose Dodecahydrate. Carbohydr. Res. **1982**, *99* (2), 103–115.
126. Fujiwara, T.; Yamazaki, M.; Tomizu, Y.; Tokuoka, R.; Tomita, K.; Matsuo, T.; Suga, H.; Saenger, W. The Crystal Structure of a New Form of Beta-Cyclodextrin Water Inclusion Compound and Thermal Properties of Beta-Cyclodextrin Inclusion Complexes. Nippon Kagaku Kaishi **1983**, *2*, 181–187.
127. Yamamoto, M.; Aritomi, H.; Irie, T.; Hirayama, F.; Uekama, K. Biopharmaceutical Evaluation of Maltosyl Beta-Cyclodextrin as a Parenteral Drug Carrier. S.T.P. Pharma Sci. **1991**, *1* (6), 397–402.
128. Yamamoto, M.; Yoshida, A.; Hirayama, F.; Uekama, K. Some Physicochemical Properties of Branched Beta-Cyclodextrins and their Inclusion Characteristics. Int. J. Pharm. **1989**, *49* (2), 163–171.
129. Ou, D.; Ueda, H.; Nagase, H.; Endo, T.; Nagai, T. Some Pharmaceutical Properties of 2,3,6-Partially Methylated-β-Cyclodextrin and its Solubilizing and Stabilizing Abilities. Drug Dev. Ind. Pharm. **1994**, *20* (12), 2005–2016.
130. Tsuchiyama Y.; Sato M.; Yagi Y.; Ishikura, T. Partially Methylated Cyclodextrins and Process for the Producing the Same. US Patent 4,746,734, 1988.
131. Harata K. Macrocyclic Conformation of Methylated Cyclodextrins Minutes of the 5th International Symposium on Cyclodextrins, Duchene, D. Editions de Santé; Paris, 1990; 77–81.
132. Irie, T.; Fukunaga, K.; Yoshida, A.; Uekama, K.; Fales, H.M.; Pitha, J. Amorphous Water-Soluble Cyclodextrin Derivatives: 2-Hydroxyethyl, 3-Hydroxypropyl, 2-Hydroxyisobutyl, and Carboxamidomethyl Derivatives of Beta-Cyclodextrin. Pharm. Res. **1988**, *5* (11), 713–717.
133. Pitha, J.; Milecki, J.; Fales, H.; Pannell, L.; Uekama, K. Hydroxypropyl- β-Cyclodextrin: Preparation and Characterization; Effects on Solubility of Drugs. Int. J. Pharm. **1986**, *29* (1), 73–82.
134. Pitha, J.; Rao, C.T.; Lindberg, B.; Seffers, P. Distribution of Ssubstituents in 2-Hydroxypropyl Ethers of Cyclomaltoheptaose. Carbohydr. Res. **1990**, *200*, 429–435.
135. Yoshida, A.; Arima, H.; Uekama, K.; Pitha, J. Pharmaceutical Evaluation of Hydroxyalkyl Ethers of Beta-Cyclodextrins. Int. J. Pharm. **1988**, *46* (3), 217–222.
136. Brandt, L.; Feicht, U.H. Water-Soluble Mixed Ether of Beta-Cyclodextrin. EP Patent 146, 841, 1985.
137. Brauns, U.; Mueller, B.W.W. Beta-Cyclodextrins Inclusion Compounds for Pharmaceuticals. DE Patent 3, 346, 123, 1985.
138. Muller, B.W.W. Preparation of Gamma-Cyclodextrins Ethers and their Use as Complexants for Chemicals and Pharmaceuticals. EP Patent 197, 571, 1986.
139. Pitha, J. Preparation of Inclusion Compounds of Cyclodextrins Ethers with Lipophilic Drugs. US Patent 4, 727, 064, 1988.
140. Lindberg, B.; Lindberg, J.; Pitha, J.; Rao, C.T.; Harata, K. Synthesis of Some 2-O-(2-Hydroxyalkyl) and 2-O-(2,3-Dihydroxyalkyl) Derivatives of Cyclomaltoheptaose. Carbohydr. Res. **1991**, *22*, 113–119.
141. Parmerter, S.M.; Allen, E.E., Jr.; Hull, G.A. Cyclodextrins With Anionic Properties. US Patent 3, 426, 011 1969.
142. Lammers, J.N.J.J.; Koole, J.L.; Hurkmans, J. Properties of Cyclodextrins. VI. Water Soluble Cyclodextrins Derivatives. Preparation and Analysis. Staerke **1971**, *23* (5), 167–171.
143. Luna, E.; Tait, R.J.; Thompson, D.O.; Stella, V.J.; Stobaugh, J.F. Evaluation of the Utility of Capillary Electrophoresis (CE) for the Analysis of Modified Cyclodextrins Mixtures Proceedings of 7th International Symposium on Cyclodextrins. Osa, T., Komiyama Printing Co. Ltd.: Tokyo 1994; 198–201.

144. Olivier, P.; Verwaerde, F.; Hedges, A.R. Subchronic Toxicity of Orally Administered Beta-Cyclodextrins in Rats. J. Am. Coll. Toxicol. **1991**, *10* (4), 407–419.
145. Koizumi, K.; Kubota, Y.; Okada, Y.; Utamura, T. Microanalyses of Beta-Cyclodextrins in Plasma by High-Performance Liquid Chromatography. J. Chromatog. **1985**, *341* (1), 31–41.
146. Gerloczy, A.; Fonagy, A.; Keresztes, P.; Perlaky, L.; Szejtli, J. Absorption, Distribution, Excretion and Metabolism of Orally Administered 14C-β-Cyclodextrin in Rat. Arzneim.-Forsch. **1985**, *35* (7), 1042–1047.
147. Szabo, P.; Ferenczy, T.; Serfozo, J.; Szejtli, J.; Liptak, A. Absorption and Elimination of Cyclodextrin Derivatives by Rabbits and Rats Procedings of 1st International Symposium on Cyclodextrins, Reidel: Dordrecht, The Netherlands 1982; 115–122.
148. Irie, T.; Tsunenari, Y.; Uekama, K.; Pitha, J. Effect of Bile on the Intestinal Absorption of Alpha-Cyclodextrins in Rats. Int. J. Pharm. **1988**, *43* (1/2), 41–44.
149. De Bie, A.; Van Ommen, B.; Bar, A. Disposition of [C-14]Gamma-Cyclodextrin in Germ-Free and Conventional Rats. Regul. Toxicol. Pharmacol. **1998**, *27* (2), 150–158.
150. Szathmary, S.C.; Seiler, K.U.; Luhmann, I.; Huss, H.J. Pharmacokinetic Behavior and Absolute Bioavailability of Hydroxypropyl Beta-Cyclodextrin after Increasing Doses in Volunteers Minutes of the 5th International Symposium Cyclodextrin. Duchene, D., Editions de Santé: Paris, 1990; 535–540.
151. Flourie, B.; Molis, C.; Achour, L.; Dupas, H.; Hatat, C.; Rambaud, J.C. Fate of Beta-Cyclodextrin in the Human Intestine. J. Nutr. **1993**, *123* (4), 676–680.
152. Antenucci, R.N.; Palmer, J.K. Enzymic Degradation of α And Beta-Cyclodextrins by Bacteroides of the Human Colon. J. Agric. Food Chem. **1984**, *32* (6), 1316–1321.
153. Antlsperger, G. New Aspects in Cyclodextrin Toxicology Minutes 6th International Symposium on Cyclodextrins Hedges, A.R., Ed.; Editions de Santé: Paris, 1992; 277–283.
154. Monbaliu, J.; Van Beijsterveldt, L.; Meuldermans, W.; Szathmary, S.; Heykants, J. Disposition of Hydroxypropyl Beta-Cyclodextrin in Experimental Animals. *Minutes 5th International Symposium on Cyclodextrins*; Duchene, D., Ed.; Editions de Santé: Paris, 1990; 514–517.
155. Gerloczy, A.; Antal, S.; Szatmari, I.; Muller-Horvath, R.; Szejtli, J. Absorption, Distribution and Excretion of Carbon-14 Labeled Hydroxypropyl Beta-Cyclodextrin in Rats Following Oral Administration. *Minutes 5th International Symposium on Cyclodextrins*; Duchene, D., Ed.; Editions de Santé: Paris, 1990; 507–513.
156. Szatmari, I.; Vargay, Z. Pharmacokinetics of Dimethyl-β-Cyclodextrin in Rats. *Proceedings of the 4th International Symposium on Cyclodextrins*; Huber, O., Szejtli, J., Eds.; Kluwer Academic Publishers: Dordrecht Netherlands, 1988; 407–413.
157. Uekama, K.; Hirayama, F.; Irie, T. Pharmaceutical Uses of Cyclodextrin Derivatives. *High Performance Biomaterials, A Comprehensive Guide to Medical and Pharmaceutical Applications*; Szycher, M., Ed.; Technomic: Lancaster, PA, 1991; 789–806.
158. Frijlink, H.W.; Visser, J.; Hefting, N.R.; Oosting, R.; Meijer, D.K.F.; Lerk, C.F. The Pharmacokinetics of Beta-Cyclodextrins and Hydroxypropyl-β-Cyclodextrins in the Rat. Pharm. Res. **1990**, *7* (12), 1248–1252.
159. Irie, T.; Uekama, K. Pharmaceutical Applications of Cyclodextrins. III. Toxicological Issues and Safety Evaluation. J. Pharm. Sci. **1997**, *86* (2), 147–162.
160. Mesens, J.L.; Putteman, P.; Verheyen, P. Pharmaceutical Applications of 2-Hydroxypropyl Beta-Cyclodextrin. *New Trends in Cyclodextrins and Derivatives*; Duchêne, D., Ed.; Editions de Santé: Paris, 1991; 369–407.
161. Yamamoto, M.; Aritomi, H.; Irie, T.; Hirayama, F.; Uekama, K. Pharmaceutical Evaluation of Branched Beta-Cyclodextrins As Parenteral Drug Carriers. *Minutes 5th International Symposium on Cyclodextrins*; Duchene, D., Ed.; Editions de Santé: Paris, 1990; 541–544.
162. French, D. The Schardinger Dextrins. Adv. Carbohydr. Chem. **1957**, *12*, 189–260.
163. Anderson, G.H.; Robbins, F.M.; Domingues, F.J.; Moores, R.G.; Long, C.L. The Utilization of Schardinger Dextrins by the Rat. Toxicol. Appl. Pharm. **1963**, *5*, 257.
164. Szejtli, J. Chemistry and Preparation of Cyclodextrins. *Cyclodextrins and Their Inclusion Complexes*; Akadémiai Kiadó: Budapest Hungary, 1982; 41.
165. Szejtli, J.; Sebestyen, G. Resorption, Metabolism and Toxicity Studies on the Peroral Application of Beta-Cyclodextrin. Starch/Staerke **1979**, *31* (11), 385–389.
166. Gergely, V.; Sebestyen, G.; Virag, S. Toxicity Studies of β-Cyclodextrin. *Proceedings of 1st International Symposium on Cyclodextrins;* Szjetli, J., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1982; 109–113.
167. Bellringer, M.E.; Smith, T.G.; Read, R.; Gopinath, C. Olivier, P. H. β-Cyclodextrin: 52-Week Toxicity Studies in the Rat and Dog. Food Chem. Toxicol. **1995**, *33* (5), 367–376.
168. Lina, B.A.R.; Bar, A. Subchronic Oral Toxicity Studies With Gamma-Cyclodextrin in Rats. Regul. Toxicol. Pharmacol. **1998**, *27* (2), 178–188.
169. Til, H.P.; Bar, A. Subchronic (13-Week) Oral Toxicity Study of Gamma-Cyclodextrin in Dogs. Regul. Toxicol. Pharmacol. **1998**, *27* (2), 159–165.
170. Frank, D.W.; Gray, J.E.; Weaver, R.N. Cyclodextrin Nephrosis in the Rat. Am. J. Pathol. **1976**, *83* (2), 367–382.
171. Frijlink, H.W.; Eissens, A.C.; Hefting, N.R.; Poelstra, K.; Lerk, C.F.; Meijer, D.K.F. The Effect of Parenterally Administered Cyclodextrins on Cholesterol Levels in the Rat. Pharm. Res. **1991**, *8* (1), 9–16.
172. Sharma, A.; Janis, L.S. Lipoprotein-Cyclodextrins Interaction. Clin. Chim. Acta **1991**, *199* (2), 129–137.
173. Matsuda, K.; Mera, Y.; Segawa, Y.; Uchida, I.; Yokomine, A.; Takagi, K. Acute Toxicity Study of Gama-Cyclodextrin (γ-Cyclodextrin) in Mice and Rats. Oyo Yakuri **1983**, *26* (2), 287–291.
174. Schmid, G. Preparation and Application of Gamma-Cyclodextrin. *New Trends in Cyclodextrins and Derivatives*; Duchêne, D., Ed.; Editions de Santé: Paris, 1991; 27–54.
175. Donaubauer, H.H.; Fuchs, H.; Langer, K.H.; Bar, A. Subchronic Intravenous Toxicity Studies with Gamma-Cyclodextrin in Rats. Regul. Toxicol. Pharmacol. **1998**, *27* (2), 189–198.

176. Irie, T.; Otagiri, M.; Sunada, M.; Uekama, K.; Ohtani, Y.; Yamada, Y.; Sugiyama, Y. Cyclodextrin-Induced Hemolysis and Shape Changes of Human Erythrocytes in Vitro. J. Pharmacobio-Dyn. **1982**, *5* (9), 741–744.
177. Garay, R.P.; Feray, J.C.; Fanous, K.; Nazaret, C.; Villegas, M.J.; Letavernier, J.F. A New Approach for the in Vitro Evaluation of Cyclodextrin Effects on Cellular Membranes of Human Cells. *Minutes 7th International Symposium on Cyclodextrins*; Tetsuo, O., Ed.; Komiyama Printing Co., Ltd.: Tokyo, 1994; 373–376.
178. Leroy-Lechat, F.; Wouessidjewe, D.; Andreux, J.P.; Puisieux, F.; Duchene, D. Evaluation of the Cytotoxicity of Cyclodextrins and Hydroxypropylated Derivatives. Int. J. Pharm. **1994**, *101* (1/2), 97–103.
179. Bar, R.; Ulitzur, S. Bacterial Toxicity of Cyclodextrins: Luminous Escherichia Coli as a Model. Appl. Microbiol. Biotechnol. **1994**, *41* (5), 574–577.
180. Saarinen-Savolainen, P.; Jarvinen, T.; Araki-Sasaki, K.; Watanabe, H.; Urtti, A. Evaluation of Cytotoxicity of Various Ophthalmic Drugs, Eye Drop Excipients and Cyclodextrins in an Immortalized Human Corneal Epithelial Cell Line. Pharm. Res. **1998**, *15* (8), 1275–1280.
181. Serfozo, J.; Szabo, P.; Ferenczy, T.; Toth-Jakab, A. Renal Effects of Parenterally Administered Methylated Cyclodextrins on Rabbits *Proceedings of 1st International Symposium on Cyclodextrins*. Szejtli, J.D., Ed.; Reidel Publishing Co.: Dordrecht, The Netherlands; 1982; 123–132
182. Anderson, W.R.; Calderwood-Mays, M.; Brewster, M.E.; Bodor, N. The Effects of Chemically-Modified Cyclodextrins on the Renal Histology of the Sprague-Dawley Rat. *Minutes 6th International Symposium on Cyclodextrins*; Hedges, A.R., Ed.; Editions de Santé: Paris, 1992, 288–291.
183. Encapsin, HPB: R81216 Hydroxypropyl-β-Cyclodextrin—A Real Solution for Real Drug Delivery Problems. Product Literature from Janssen Biotech N. V. Drug Delivery Systems. Lammerdries 55, B-2250 Olen Belgium, 1992.
184. Vollmer, U.; Müller, B.W.; Peeters, J.; Mesens, J.; Wilffert, B.; Peters, T. A Study of the Percutaneous Absorption-Enhancing Effects of Cyclodextrin Derivatives in Rats. J. Pharm. Pharmacol. **1994**, *46* (1), 19–22.
185. Park, G.-B.; Seo, B.; Ann, H.-J.; Rho, H.-G.; Onn, Y.-S.; Lee, K.-P. Enhanced Nasal Absorption of Ketoconazole by Inclusion With Cyclodextrin. Yakche Hakhoechi. **1994**, *24* (2), 95–104.
186. Captisol®. *Sulfobutyl Ether β-Cyclodextrin*; Product Literature from CyDex, Inc.: 12980 Metcalf Ave., Suite 470, Overland Park KS 66213, 2000, CDinfo@cydexinc.com..
187. Coussement, W.; Van Cauteren, H.; Vandenberghe, J.; Vanparys, P.; Teuns, G.; Lampo, A.; Marsboom, R. Toxicological Profile of Hydroxypropyl Beta-Cyclodextrin (HP Beta-Cyclodextrins) in Laboratory Animals. *Minutes 5th International Symposium on Cyclodextrins*; Duchene, D., Ed.; Editions de Santé: Paris, 1990; 522–524.
188. Brewster, M.E.; Bodor, N. Parenteral Safety and Uses of 2-Hydroxypropyl Beta-Cyclodextrins. *Minutes 5th International Symposium on Cyclodextrins*; Duchene, D., Ed.; Editions de Santé: Paris, 1990; 525–534.
189. Ladola, A.; Abbott, D.; George, C.; Guzzie, P.; Nahas, K.; Provost, J.-P. Safety Studies With Captisol. Symposium on Pharmaceutical Applications of Cyclodextrins, Lawrence: KS, June-July 29-2 1997.
190. Van Cauleren, H.; Lampo, A.; Lammens, L.; Benze, H.; Coussement, W.; Vandenberghe, J. Monitoring Pharmacodynamic Effects in Toxicology Studies. *Excerpts From a Workshop on The Use of Pharmacology Studies in Drug Safety Assessment—Present Situation and Future Perspectives*; Sundwall, A.; Johansson, B.; Lindbom, L.-O.; Lindgren, E.; Sjöberg, P., Eds.; 1994; 101–106.
191. Sundaram, S.; Dayan, A.D. Effects of a Cholecystokinin Receptor Antagonist on Rat Exocrine Pancreatic Response to Raw Soya Flour. Human Exp. Toxicol. **1991**, *10*, 179–182.
192. Expert Working Group (Safety) of ICH. FDA Guidance for Industry ICH-S1C: Dose Selection for Carcinogenicity Studies of Pharmaceuticals, Drug Information Branch, HFD-210, CDER, 5600 Fishers Lane, Rockville, MD 20857 accessed Sept. 22, 1998, http://www.fda.gov/CDER /guidance/ index.htm.
193. Waalkens-Berendsen, D.H.; Smits-Van Prooije, A.E.; Bar, A. Embryotoxicity and Teratogenicity Study with Gamma-Cyclodextrin in Rabbits. Regul. Toxicol. Pharmacol. **1998**, *27* (2), 172–177.
194. Barrow, P.C.; Olivier, P.; Marzin, D. The Reproductive and Developmental Toxicity Profile of Beta-Cyclodextrin in Rodents. Rep. Toxicol. **1995**, *9* (4), 389–398.
195. Möller, H.; Oeser, W.H. Drug Master Files, Global Harmonization of Quality Standards, Wissenschaftlich Verlagsgesellschaft MbH: Stuttgart, 1992; 213.
196. *The Japanese Pharmaceutical Excipients Council. Japanese Pharmaceutical Excipients 1993.* Yakuji Nippo, Ltd.: Tokyo, 1994; 415.
197. Ed. *United States Pharmacopeia 24/National Formulary 19*, United States Pharmacopeial Convention, Inc.: Rockville, MD, 1999; 24/19, 2569.
198. Janssen Research Foundation, Itraconazole Oral Solution, NDA 020657, Food and Drug Administration, Freedom of Information Staff (HFI-35),5600 Fishers Lane, Rockville MD 20857, http://www.fda.gov/.